Mahendra Rai · Alka Yadav *Editors*

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Preface

Neurodegenerative (ND) diseases comprise a range of conditions that primarily affect the neurons in the human brain. Neurons are the building blocks of our brain, which normally don't reproduce or replace themselves if they get damaged. ND diseases include Parkinson's disease, Alzheimer's disease, and Huntington's disease. The most problematic feature of ND diseases is that they are incurable and result in the progressive degeneration of neuron cells. The major cause of ND diseases is related to genetic mutation; apart from this, protein misfolding, DNA damage, apoptosis, mitochondrial dysfunction, and programmed cell death are the other causes of ND diseases. A number of ND disorders have been thoroughly examined, but successful, early diagnoses and treatment have been limited. The key reason for this is the blood–brain barrier that prevents the penetration of the majority of drugs and agents to effectively treat the disorders.

Nanobiotechnology as an emerging tool has the potential to play a pivotal role to improve the understanding and treatment of neurodegenerative diseases. Different types of nanomaterials can be implemented for diagnosis, drug delivery, and treatment of neurodegenerative diseases. Engineered nanoparticles are materials with dimension 1–100 nm. Metal nanoparticles showcase an innovative and promising approach to potentially solve problems related to ND diseases. Due to their smaller size, nanoparticles are able to interact with biological systems at a molecular level. The smaller sized nanoparticles can also cross the blood–brain barrier. Hence, the physical, chemical, and biological properties of nanoparticles can be utilized for diagnosis, therapy, tissue engineering, regeneration, and drug delivery. Similarly, exosomes are also evolving as therapeutic nanobiomaterials for drug delivery in neurodegenerative diseases as they have the ability to cross the blood–brain barrier. Moreover, in recent years, the field of nanobiosensors is growing quickly, and with the help of nanotechnology, it is possible to develop higher sensitivity for nanobiosensors. This area of research is attracting scientists in general and medical experts in particular. In this book, a brief overview of the different ND diseases and advancements in nanobiotechnology for the treatment of ND is discussed by eminent contributors.

This book is beneficial for a wide range of readers including nanotechnologists, biotechnologists, pharmacists, medical professionals, bioengineers, biochemists, and researchers who are involved in the field of research on neurodegenerative diseases.

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Chapter 1
Neurodegenerative Diseases: The Real Problem and Nanobiotechnological Solutions

Mahendra Rai, Alka Yadav, Avinsh P. Ingle, Anatoly Reshetilov, María José Blanco-Prieto, and Chistiane M. Feitosa

Abstract Neurodegenerative diseases are now the most debilitating disorders affecting the human population. In recent times, neurodegenerative diseases have become the fourth leading cause of death after heart disease, cancer, and stroke. Neurodegenerative diseases affect the thinking, skilled movements, feelings, cognitive behavior, and memory of a person, resulting in short-term and long-term impairment and disabilities. Neurodegenerative diseases include serious disorders like Alzheimer's disease, Parkinson's disease, dementia, and other rare disorders like amyotrophic lateral sclerosis, Huntington's disease, and prion diseases. Although a

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century has passed since the discovery of neurodegenerative diseases, there is still a need for more diagnostic approaches and effective cure. The emerging field of nanotechnology promises new techniques to solve some of the challenges in this field. Nanotechnological tools enable drugs to cross the blood–brain barrier and target the site of action in a specific manner. The new generation nanoparticles could also be useful in the treatment of brain diseases. In the present chapter, we explain the way ahead for nanotechnology for the treatment of neurodegenerative disorders.

Keywords Neurodegenerative diseases · Alzheimer's · Parkinson's · Nanotechnology

1.1 Introduction

Neurodegenerative diseases take in an assortment of clinical symptoms including selective dysfunction and loss of synapses, neurons, protein aggregates, and neuroinflammation that cause devastating changes in behavior and cognition (Modi et al. 2010; Gitler et al. 2017). Neurodegenerative diseases include Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), frontotemporal dementia, Huntington's disease, and prion disease, which affect enormous numbers of people globally (Re et al. 2012; Muthu Lakshmi et al. 2017; Poupot et al. 2018). According to recent studies, around 30 million people suffer from Alzheimer's disease worldwide (Sheikh et al. 2013; Nguyen et al. 2017). Hence, the social, economic, and health impact of neurodegenerative diseases is very considerable (Re et al. 2012; Tosi et al. 2015). But the mechanism of neurodegenerative diseases is still an unsolved mystery, and we still lack clinical therapeutics for their treatment (Spuch et al. 2012; Vieira and Gamarra 2018). The possible reasons for this failure in the treatment and management of neurodegenerative diseases include the presence of the blood–brain barrier, which works as a defense system and prevents the passage of drugs, so that these work only on the symptoms of the disease. Moreover, the diagnostic markers used are unvalidated and work only on probable or possible diagnosis (Sharma et al. 2015; Niu et al. 2018; Torre and Cena 2018; Serra et al. 2019).

Recent advances in nanotechnology are expected to have a major impact on neurological research, leading to the development of newer and more efficient therapeutic modalities. Nanotechnology employs engineered nanomaterials to produce nanoscale devices that interact with biological systems at a molecular level (Bhatt et al. 2011; Re et al. 2012; Kasinathan et al. 2015; Rahman 2018; Ramanathan et al. 2018). These nanotechnological devices can stimulate, respond, and interact with target cells and tissues to bring about the preferred physiological response while lowering undesirable side effects (Kabanov and Gendelmen 2007; Nazem and Ali 2008; Win-Shwe and Fujimaki 2011; Kaushik et al. 2018). Most importantly, nanotechnology offers ways to modulate complex biological systems with higher selectivity. Nanoengineered particles as nanodrugs possess the capacity to cross the blood–brain barrier and also show decreased invasiveness (Sheikh et al. 2013; Silva

et al. 2017; Ran and Xue 2018). Therefore, in this chapter, we propose the use of nanotechnological solutions for the treatment of neurodegenerative disorders given the lack of effective clinical therapeutics.

1.2 Neurodegenerative Diseases

Neurodegenerative diseases occur when nerve cells in the brain lose their functionality or undergo untimely death (Re et al. 2012; Alyautdin et al. 2014; Furtado et al. 2018). Although there are treatments associated with neurodegenerative diseases which lower their physical and mental impact, currently there is no specific treatment that can cure the disease or slow down its progression (Long et al. 2006; Saraiva et al. 2016; Liu et al. 2017; Torre and Cena 2018). The most common neurodegenerative diseases are Alzheimer's disease and Parkinson's disease, others include Huntington's disease, ALS, motor neuron disease, and prion disease (Yu and Lyubchenko 2009; Tosi et al. 2015; Boonruamkaew et al. 2017; Lovisolo et al. 2018).

The risk of being affected by a neurodegenerative disease increases dramatically with age. Studies suggest that a large population will be affected by neurodegenerative diseases in the coming decades, which points to a critical need to study their causes and develop new approaches for their treatment and prevention (Baratchi et al. 2009; Adams et al. 2010; Erickson and Banks 2013; Dai et al. 2018).

1.2.1 Alzheimer's Disease

Alzheimer's disease is associated with prominent cognitive deficits (Dam and De Deyn 2011; Karthivashan et al. 2018). It is one of the most common causes of dementia in elderly people. At an early stage, a person suffering from Alzheimer's disease has limited forgetfulness with trouble in memory imprinting, which leads to short-term memory loss and finally to long-term memory deficits (Nazem and Ali 2008; Mehta et al. 2012; Sharma et al. 2015; Torre and Cena 2018). These symptoms lead to difficulty in thinking, remembering, and reasoning and to behavioral disabilities that interfere with the person's daily life and activities (Re et al. 2012; Tosi et al. 2015; Saraiva et al. 2016). Thus, Alzheimer's disease eventually leads to a dementia syndrome in which it firstly affects the person's functioning and in severe stages leads to complete dependency on others to carry out even the most basic chores (Brambilla et al. 2011; Alyautdin et al. 2014; Gitler et al. 2017).

The scientific history of Alzheimer's disease goes back to the year 1906, when Dr. Alois Alzheimer studied the brain tissues of a woman who died an untimely death from mental illness. He found many abnormal clumps and tangled bundles of fibers in the brain tissues (Hippius and Neudorfer 2003). These plaques and tangles are considered to be the main characteristic of the disease (Adams et al. 2010; Brambilla et al. 2011; Boonruamkaew et al. 2017). The first sign of Alzheimer's

disease is definitely linked to memory problems, while other signs include difficulty in word finding, impaired reasoning or judgment, eyesight issues, etc. (Dam and De Deyn 2011; Erickson and Banks 2013; Karthivashan et al. 2018). As the disease progresses, there is greater memory loss, trouble in handling cash and paying, wandering and getting lost, difficulty in completing daily chores, and personality and behavioral changes. In further stages, control over language is lost, confusion grows, and patients have problems recognizing family and friends (Kim et al. 2008; Masserini 2013; Lovisolo et al. 2018). Finally, the plaques and tangles spread all over the brain and the patient becomes completely dependent on others. The treatment of Alzheimer's disease is very complex as there is no one drug for handling the disease successfully (Kirkitadze et al. 2002; Modi et al. 2010; Rahman 2018).

1.2.2 Parkinson's Disease

Parkinson's disease currently ranks second after Alzheimer's disease and is characterized by progressive deterioration of motor functions due to confiscation of dopamine-releasing neurons (Adhikary et al. 2015; Barcia et al. 2017). In Parkinson's disease, neurons lose their functionality which results in cognition impairment and forgetfulness, shaking, stiffness, difficulty in walking, balancing, and coordination (Yu and Lyubchenko 2009; Sousa et al. 2010; Nguyen et al. 2017). The signs of Parkinson's disease begin slowly and worsen with the passing of time. Both men and women above the age of 50 may be affected by Parkinson's disease (Kaushik et al. 2018).

Parkinson's disease mainly occurs when the nerve cells or neurons of the brain that control the movement of the body get damaged or die (Yu and Lyubchenko 2009; Adhikary et al. 2015; Barcia et al. 2017). This means that the release of dopamine is reduced, which eventually results in difficulty in body movements. People suffering from Parkinson's disease also lose nerve endings that produce norepinephrine. At present there are no specific treatments for Parkinson's disease, and the drugs, surgeries, and therapies prescribed can only relieve the symptoms of the disease (Benabid et al. 2009; Giordano et al. 2011; Cacciatore et al. 2012; Carradori et al. 2017). Among the available treatment approaches used for Parkinson's disease, use of protein like human glial cell line-derived neurotrophic factor (hGDNF) was found to be promising. Ansorena et al. (2013) demonstrated a quick and simple method to produce a high amount of purified hGDNF using a mammalian cell-derived system.

1.3 Nanomaterials and Biomedical Applications

Nanotechnology refers to the utilization and application of materials at the nanoscale level (1–100 nm). Nanoparticles can stimulate, respond to, and interact with the target cells to produce desired physiological results while minimizing the side

effects (Baratchi et al. 2009; Modi et al. 2010; Kumari et al. 2010; Safari et al. 2016; Dai et al. 2018). At the present time, different types of nanomaterials like nanotubes, nanofibers, quantum dots, and nanoparticles are used for biomedical applications. Nanoparticles are materials with at least one dimension less than 100 nm. Based on their structure, they can be categorized into zero dimensional, which involves nanoparticles and quantum dots; one dimensional, which includes nanofibers, nanotubes, and nanowires; and two dimensional, consisting of graphene (Bhatt et al. 2011; Teleanu et al. 2018a).

In recent years, nanoparticles have been used in a variety of biomedical applications for drug delivery, biosensors, bioimaging, etc. (Khatoon et al. 2009; Masserini 2013; Lovisolo et al. 2018). Nanoparticles have also been used in the imaging and treatment of brain diseases, including brain tumors, brain cancer, and central nervous system disorders (Re et al. 2012; Saraiva et al. 2016; Poupot et al. 2018). In this chapter, special emphasis has been placed on the role of nanotechnology in the diagnosis and treatment of neurodegenerative diseases.

1.4 Role of Nanotechnology in the Treatment of Neurodegenerative Diseases

Nanomedicine is a subdivision of nanotechnology featuring its medical applications (Ran and Xue 2018). Neuronanomedicine refers to the engineered nanomaterials designed as drugs for neurodegenerative disorders (Kabanov and Gendelmen 2007; Tosi et al. 2015). Teleanu et al. (2019) recently discussed an emerging role for neuronanomedicine as a new field that constitutes a link between neurological sciences and nanotechnology. It is a promising field for the diagnosis and treatment of central nervous system (CNS) disorders.

The CNS is the most complex system of the body, and the disorders related to the CNS are equally complex (Sheikh et al. 2013; Sharma et al. 2015). Treatments related to brain disorders are still insufficient due to the presence of the blood–brain barrier in the CNS. The blood–brain barrier is primarily composed of brain endothelial cells which are lined with microvessels and capillaries in the brain and are knitted very tightly with junctions leaving no gaps between the cells (Fig. 1.1) (Furtado et al. 2018; Teleanu et al. 2018b). The brain base endothelial cells are enclosed by basal lamina, which consist of fibronectin, type IV collagen, laminin, and heparin sulfate (Hawkins and Davis 2005; Kasinathan et al. 2015). Thus, only a limited amount of drug can enter the CNS, which is insufficient for the treatment of these highly complex disorders (Teleanu et al. 2018a; Mendes et al. 2018).

Nanotechnology offers a ray of hope for the treatment of CNS disorders due to its novel strategy of drug delivery (Shilo et al. 2015; Rahman 2018; Sun et al. 2019). Due to their extremely small size, nanoparticles can easily cross the blood–brain barrier and deliver drug to the target tissues without invading the normal tissues (Chertok et al. 2008; Spuch et al. 2012; Silva et al. 2017; Niu et al. 2018). Thus,

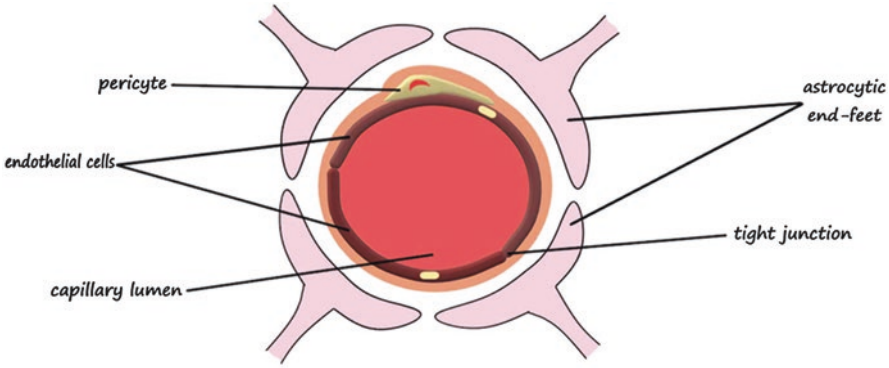

Fig. 1.1 Various types of nanocarriers commonly used for diagnosis and treatment of the most prevalent neurological disorders (Adapted from Teleanu et al. (2019); an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license which permits unrestricted use, distribution, and reproduction in any medium)

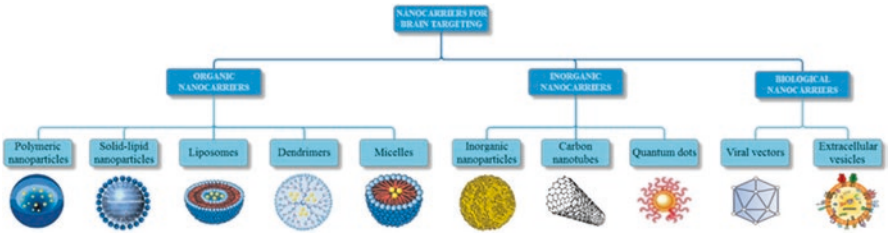

Fig. 1.2 Structural components of blood–brain barrier (Adapted from Teleanu et al. (2018b); an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium)

nanotechnology, with its wide range of biomedical applications, could play a vital role in the diagnosis and treatment of various neurodegenerative diseases (Garbayo et al. 2014). Recently, Torres-Ortega et al. (2019) reviewed the latest advances in emerging role of micro- and nanomedical approaches for repairing dopaminergic neurons. According to them, micro- and nanoparticles can be used as powerful tools for the administration of various drugs to the brain, enabling the development of new strategies against the neurodegenerative diseases like Parkinson's. Figure 1.2 represents various types of nanomaterials that can be used as neuronanomedicines in the form of nanocarriers in the diagnosis and treatment of the most prevalent neurological disorders.

Drug delivery to the CNS for the treatment of neurodegenerative diseases is a complex issue (You et al. 2018; Samaridou and Alonso 2018). The most widely exploited use of nanoparticles is in the field of drug delivery, in which engineered nanoparticles are used to deliver drugs to the target cells. Baratchi et al. (2009) described the role of nanoparticles for drug delivery using carbon nanotubes,

nanowires, and metal nanoparticles. The authors explain that the small size of nanoparticles makes them the ideal choice for drug delivery across the blood–brain barrier. Trapani et al. (2011) aimed to characterize nanoparticles composed of chitosan (CS) and evaluate their potential for brain delivery of the neurotransmitter dopamine (DA). For their study, CS-based nanoparticles were incubated with DA at two different concentrations giving rise to nanocarriers described as DA/CSNPs and DA/CSNPs. The X-ray photoelectron spectroscopy (XPS) analysis confirmed that DA was adsorbed onto the external surface of such nanoparticles. Also, the cytotoxic effect of the CSNPs and DA/CSNPs was assessed using the MTT test, and it was reported that the nanovectors are less cytotoxic than the neurotransmitter DA after 3 h of incubation time. The transport studies across MDCKII-MDR1 cell line denoted that DA/CSNPs brought about a significant transport-enhancing effect. Measurement of reactive oxygen species (ROS) showed low DA/CSNP neurotoxicity after 3 h. Also, the in vivo brain microdialysis experiments in rats demonstrated that intraperitoneal acute administration of DA/CSNPs (5) (6–12 mg/kg) induced a dose-dependent increase in striatal DA output. Hence, chitosan nanoparticles seemed to offer an interesting technological platform for DA brain delivery and could be useful for Parkinson's treatment.

Bhatt et al. (2011) developed ropinirole hydrochloride nanoparticle and studied its release profile. For the study, ropinirole hydrochloride nanosuspension stabilized by poloxamer F-68 was prepared by milling technique and later lyophilized to obtain nanoparticles using mannitol (1:1 w/v) as cryoprotectant. The nanoparticles developed were characterized for their particle size and size distribution, drug content, and % drug entrapment. An in vitro dissolution study using a dissolution bag (12,000 D) and an ex vivo study in rat ileum were carried out using 0.1 N hydrochloric acid as dissolution medium. The particle size, zeta potential, % drug content, and % drug entrapment of the nanoparticles of the ropinirole hydrochloride were found to be 282 nm ± 23, 54.9 mV ± 9.36, 98.9 ± 0.86%, and 62 ± 0.87%, respectively. The in vitro, ex vivo permeation study of ropinirole nanoparticles showed that the cumulative percentage of drug permeated was 73.7 ± 1.9% and 65.26 ± 1.1% in 24 h. From the results, it was evident that the ropinirole hydrochloride nanoparticles developed could be used as an alternative for the treatment of Parkinson's disease.

Papadimitriou et al. (2016) reported the synthesis and characterization of two different classes of polymeric nanoparticles: N-isopropylacrylamide-based thermoresponsive nanogels RM1 and P(TEGA)-b-P(D,LLA)2 nanomicelles RM2. The authors covalently linked the nanoparticles with fluorescent tags and showed that they could be internalized and tracked in neural stem cells from the postnatal subventricular zone, without this affecting their proliferation, multipotency, and differentiation characteristics up to 150 μg ml − 1. It was observed that the chemical structure of RM1 and RM2 did not appear to cause toxicity, but it did influence the loading capacity. Nanogel RM1 loaded with retinoic acid improved the solubility of drug which was released at 37 °C, resulting in an increase in the number of neurons.

Boonruamkaew et al. (2017) aimed to assess whether the newly developed redox nanoparticle (RNPN) decreases Aβ levels or prevents Aβ aggregation associated

with oxidative stress. For the study, transgenic Tg2576 Alzheimer's disease mice were used to investigate the effect of chronic ad libitum drinking of RNPN solution for 6 months, on memory and learning functions, antioxidant activity, and amyloid plaque aggregation. The results showed that RNPN-treated mice had significantly attenuated cognitive deficits regarding both spatial and nonspatial memories and reduced oxidative stress of lipid peroxide and DNA oxidation. Also, RNPN treatment increased the percent inhibition of superoxide anion and glutathione peroxidase activity and the neuronal densities in the cortex and hippocampus, but decreased the Aβ(1–40), Aβ(1–42), and gamma (γ)-secretase levels, while reduced Aβ plaque was also observed using immunohistochemistry analysis and thioflavin S staining. The authors suggested that RNPN may be a promising candidate for Alzheimer's disease therapy because of its antioxidant properties and reduction in Aβ aggregation.

Barcia et al. (2017) exploited ropinirole-loaded nanoparticles for drug delivery. The formulations were evaluated in a rotenone (RT)-induced animal model of Parkinson's disease. Daily doses of the neurotoxin rotenone (2 mg/kg) were given to male Wistar rats, which induced neuronal and behavioral changes similar to those of Parkinson's disease. Once neurodegeneration was established (15 days), animals received ropinirole in saline (1 mg/kg/day for 35 days) or encapsulated within PLGA nanoparticles (amount of NPs equivalent to 1 mg/kg/day RP every 3 days for 35 days). Brain histology and immunochemistry (Nissl-staining, glial fibrillary acidic protein, and tyrosine hydroxylase immunohistochemistry) and behavioral testing (catalepsy, akinesia, rotarod, and swim test) showed that the ropinirole-loaded nanoparticles were effective in reverting neurodegeneration in this RT-induced animal model.

Amin et al. (2017) evaluated nanoparticle-based drug delivery approach for the treatment of Alzheimer's disease. In the study, fluorescent magnetic nanoparticles were exploited for delivery to the brain cells of normal mice under functionalized magnetic field. The fluorescent magnetic nanoparticles successfully crossed the blood–brain barrier and reached the brain cells. According to Wen et al. (2017), it is possible to deliver the drugs by different routes using nanotechnology-based drug delivery systems, and it can be considered as promising tools to improve patient compliance and achieve better therapeutic outcomes in patients suffering from Alzheimer's disease. However, from the extensive literature survey, they noticed that despite extensive research, clinical activities involving nanotechnology-based drug delivery system application in research for Alzheimer's disease are lagging compared to its use for other diseases like cancers. Therefore, more scientific efforts are required toward the use of nanotechnology-based drug delivery systems for different neurodegenerative diseases including Alzheimer's disease.

Gonzalez-Carter et al. (2017) examined the effect of silver nanoparticles on the resident immune cells of the brain, the microglia. The study focused on microglia in neurodegenerative disorders such as Parkinson's disease to examine the effect of silver nanoparticles on microglial inflammation. The in vitro uptake and intracellular transformation of citrate-capped silver nanoparticles by the microglia and their effects on microglial inflammation and related neurotoxicity were examined. Analytical microscopy depicted internalization and dissolution of silver nanoparti-

cles within microglia and formation of nonreactive silver sulphide on the surface of silver nanoparticles. Moreover, silver nanoparticle treatment upregulated microglial expression of the hydrogen sulphide (H2S)-synthesizing enzyme cystathionine-γ-lyase (CSE). Furthermore, silver nanoparticles demonstrated significant anti-inflammatory effects, reducing lipopolysaccharide (LPS)-stimulated ROS, nitric oxide, and TNFα production, which translated into reduced microglial toxicity toward dopaminergic neurons. The authors concluded that intracellular silver sulphide formation resulting from CSE-mediated H2S production in microglia sequestered Ag^{+} ions released from silver nanoparticles, considerably limiting their toxicity, as well as reducing microglial inflammation and related neurotoxicity.

1.5 Mechanism of Nanoparticle Transport across the Blood–Brain Barrier

As mentioned above, the CNS is the most complicated and sensitive system of the human body. It is tightly sealed by the blood–brain barrier and the blood cerebrospinal fluid (Hawkins and Davis 2005; Erickson and Banks 2013; Furtado et al. 2018). It selectively allows small individual molecules to pass through the capillary endothelial membrane, while preventing the passage of pathogens/toxins (Engelhardt and Sorokin 2009; Gobbi et al. 2010; Erickson and Banks 2013; Alyautdin et al. 2014). However, this property of the blood–brain barrier proves a major obstacle at the time of drug delivery, since more than 98% of molecular drugs cannot pass through the blood–brain barrier (Erickson and Banks 2013; Alyautdin et al. 2014).

Most nanoparticles cannot freely pass through the blood–brain barrier and need receptor-mediated transcytosis (Fig. 1.3) (Blasi et al. 2007; Re et al. 2012; Masserini 2013). In this method, nanoparticles are functionalized with different types of ligands, such as insulin, transferring, lactoferrin, or surfactants like polysorbate 80 (Masserini 2013; Saraiva et al. 2016; Teleanu et al. 2018a). The interaction between nanoparticles bounded with ligands and the brain endothelial cells leads to plasma membrane retraction followed by formation of pinch free vesicles, which results in the release of nanoparticles to the brain parenchyma without damaging the blood–brain barrier (Tosi et al. 2015; Silva et al. 2017; Rahman 2018).

1.6 Role of Nanosized Exosomes in the Management of Neurodegenerative Diseases

Exosomes can be defined as nanosized (40–150 nm) membranous vesicles which develop from endosomes (Fig. 1.4). These are the extracellular secretions of different cells and can be recovered from body fluids, including urine, blood, saliva, milk, and cerebrospinal fluid (CSF). The quantity of exosomes changes when cells are

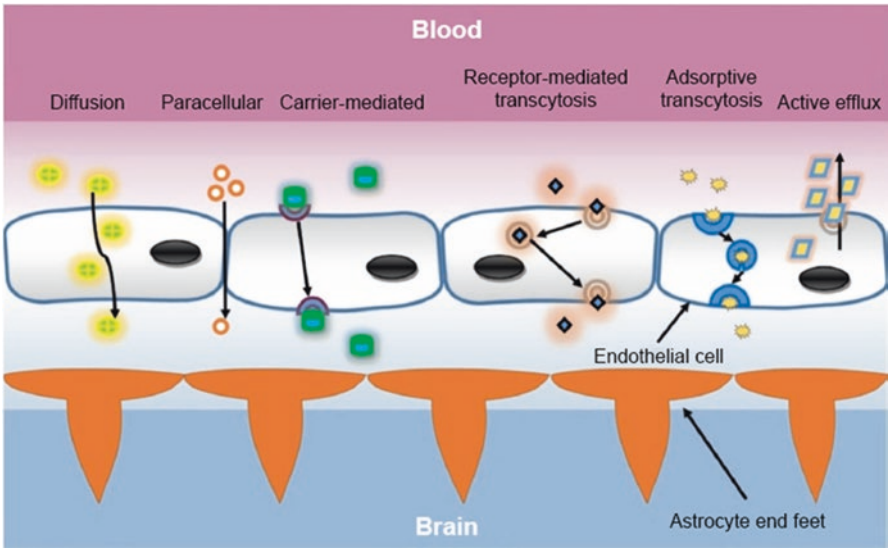

Fig. 1.3 Mechanism of transport across blood–brain barrier (Adapted from Ramanathan et al. (2018); an open access article; distributed under the Creative Commons Attribution-NonCommercial (unported, v3.0) License which permits unrestricted use, distribution, and reproduction in any medium)

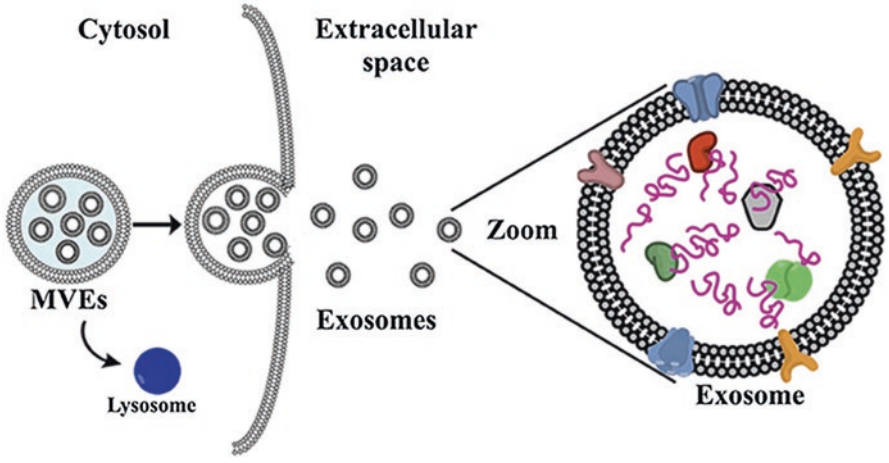

Fig. 1.4 Schematic representation of exosome biogenesis: Initially, early endosomes are formed which are further converted to multivesicular endosomes (MVEs), and later these MVEs encompass the exosomes. The MVEs can either fuse with the plasma membrane, releasing the exosomes into the extracellular matrix (see zoomed schematic), or fuse with the lysosome for degradation. Exosomes may be protein, DNA, RNA, and surface membrane proteins, which are specific to the cell of origin and are not limited to cell surface proteins (Colombo et al. 2014) [adapted from Gomez et al. (2018), an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY) which permits unrestricted use, distribution, and reproduction in any medium]

diseased. For this reason, exosomes can be used in diagnostics. The main function of exosomes is cellular communication (cell–cell signaling), transmission of pathogens between cells and elimination of cellular remains (Wu et al. 2017).

Various reports provide evidence that exosomes derived from the CNS are usually present in the CSF. During the appearance of the disease, their content changes, and therefore, these exosomes can be used as biomarkers in neurodegenerative diseases, in general, and Parkinson's disease, in particular. It has been reported that exosomes propagate toxic a-synuclein (asyn) among the cells leading to apoptosis, which is responsible for spread of asyn aggregates in brain. In other studies, the protective role of exosomes has been reported. As far as the treatment strategy for neurodegenerative diseases is concerned, exosomes can be used in drug delivery, for example, in the delivery of catalase and small-interfering RNAs (siRNA) to the brain. Pardridge (2012) reported that about 98% of the drugs that are used in CNS diseases fail to cross the blood–brain barrier. Exosomes have the ability to cross the blood–brain barrier, and hence can be used as drug delivery tools (Alvarez-Erviti et al. 2011; Zhuang et al. 2011). The use of exosomes secreted by mesenchymal stem cells has an advantage, in that their nanosize means that they can be easily administered intranasally and intravenously. Recently, Riazifar et al. (2019) studied the effect of exosomes secreted from human mesenchymal stem cells (MSCs) in the treatment of multiple sclerosis. The authors used an autoimmune encephalomyelitis (EAE) mouse model and reported that intravenous administration of exosomes secreted by MSCs IFNγ (IFNγ-Exo) is beneficial. On the basis of the results of this experiment, the authors concluded that exosomes obtained from MSCs can be used in treatment for neurodegenerative diseases.

1.7 Nanobiosensor for Rapid Detection of Neurodegenerative Diseases

In recent decades, the field of biosensors has been growing, and the application of nanotechnology has developed as one of the biggest opportunities to achieve higher sensitivity for nanobiosensors (Topkaya et al. 2016). Among the different types of nanobiosensors, those based on electrochemical methods are still the most attractive due to the advantages of cost effectiveness, easy production, and user friendliness (Kaushik et al. 2018). Alzheimer's disease is the most common form of dementia, and its early detection using reliable molecular biomarkers is believed to be best approach for control and even treatment (Azimzadeh et al. 2017). Azimzadeh et al. (2017) developed an ultrasensitive electrochemical nanobiosensor to quantify serum miR-137 as a validated biomarker of AD. Electrochemically reduced graphene oxide and gold nanowires were used to modify the surface of a screen-printed carbon electrode with the application of an intercalated label, doxorubicin. The fabrication steps were analyzed using field emission scanning electron microscopy and energy dispersive spectroscopy, as well as two reliable electrochemical methods,

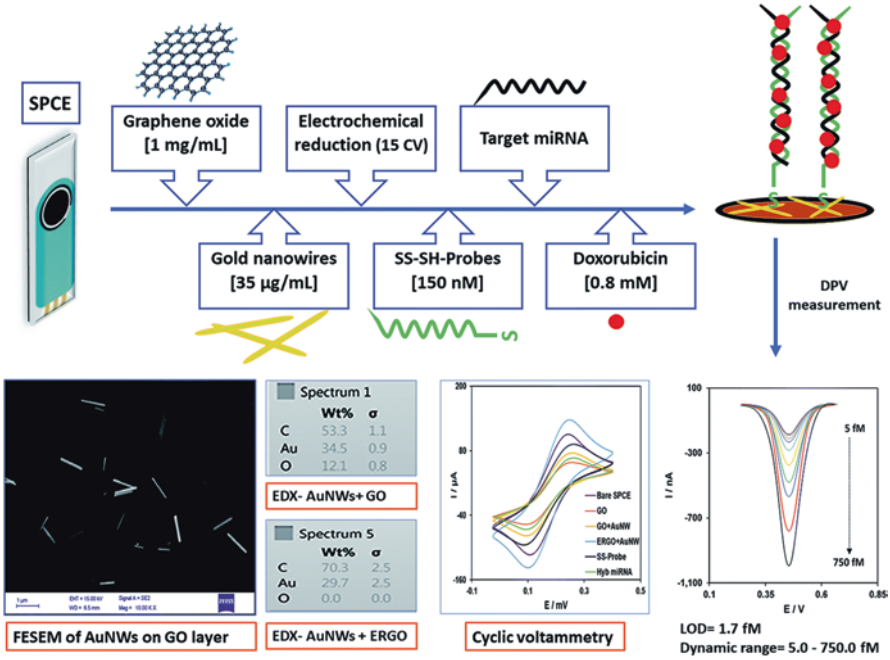

Fig. 1.5 Overview of the assembly, characterization, and working mechanism of the nanobiosensor [adapted from Azimzadeh et al. (2017); an open access article, licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported License which permits unrestricted use, distribution, and reproduction in any medium]

cyclic voltammetry and electrochemical impedance spectroscopy. The results show the very good linear range of the nanobiosensor and its limit of detection (about 1.7 fM). In addition, the nanobiosensor was found to have a good performance in specificity experiments and was able to discriminate between target oligos versus nonspecific oligos (one-base mismatch target; three-base mismatch target, nonspecific miR-21 and miR-155) very well. A recent review by Tiwari et al. (2019) discusses the possibility of developing nanobiosensors for the detection of epilepsy. The biosensors used for epilepsy are composed of a nanoenabled smart sensing platform. This diagnostic system affords an opportunity for us to gain a better understanding of this serious disease. Figure 1.5 represents the overview of the assembly, characterization, and working mechanism of the nanobiosensor.

1.8 Conclusion

Neurodegenerative diseases have become the prime focus of many research efforts, as the diagnostic methods available can only address the symptoms and not the disease process itself. In this scenario, nanotechnology offers many advantages with

exciting possibilities. Recently, nanomedicine has become one of the main branches of nanotechnology research offering drug delivery systems like polymeric nanoparticles, liposomes, and other engineered nanomaterials for diagnosis and treatment of various diseases. Due to their extremely small size, these engineered nanomaterials can cross the blood–brain barrier and allow specific and selective delivery of the drug to the target. Thus, given the current rise in the incidence of neurodegenerative diseases, various nanotechnology approaches are also gathering momentum for their treatment. However, further experiments are also necessary to achieve a better understanding of the mechanisms of nanoparticle-mediated drug transport to the brain cells, as this would enable us to improve the treatment of neurodegenerative diseases, decrease drug doses, reduce side effects, and increase patients' life expectancy.

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Chapter 2
Nanotechnology at the Rescue of Neurodegenerative Diseases: Tools for Early Diagnostic

Jean-Michel Rabanel, Morgane Perrotte, and Charles Ramassamy

Abstract Fundamental causes of neurodegenerative diseases (NDDs) such as Alzheimer's (AD) and Parkinson's diseases (PD) remain a matter of debate, and the discovery of disease-modifying treatments represents a great challenge. Currently, patients with NDD have only access to actives intended to alleviate symptoms and not to target fundamental causes of the diseases. Meanwhile, in the last few years, a strong conviction has emerged from the clinical and fundamental research that an early diagnosis, even before any cognitive or motor symptoms, is the path toward improvement of the therapeutic course of these irreversible pathologies.

This urgent need for early diagnostic tests could be in a near future, at least partially addressed by advances in the therapeutic use of nanotechnology. The use of nanoparticles (NPs) in the biomedical field is mostly recognized for drug delivery applications. Nevertheless, the potential of nanotechnology is now also recognized for providing new tools to explore physiology and pathophysiology. The area of NP-assisted in vivo imaging is particularly active. Indeed, functionalized nano-objects, such as liposomes, micelles, inorganic semiconductors, metallic and polymer NPs, could have huge impact on in vivo detection of pathological targets and analysis of brain tissue in real time. In this chapter, we propose a critical overview of the use of NPs in neurosciences focused on in vivo NDD diagnostic. Emphasis will be put on in vivo recent functional study of diseased tissues using different types of NP.

Keywords Nanotechnology · Neurodegenerative diseases · Early diagnosis · Alzheimer's disease · Parkison's disease

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2.1 Introduction

Neurodegenerative diseases (NDDs) represent a social and economic burden affecting the quality-of-life of millions of people worldwide (Prince et al. 2015). Among all NDDs, Alzheimer's disease (AD) is the most frequent dementia counting for 60–70% of all cases (Winblad et al. 2016). Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), and Huntington's disease (HD) represent the most prominent NDDs. Actually, the mechanisms underlying the pathophysiology of these NDDs remain unclear. Moreover, the lack of early diagnostic tools is impeding the development of early therapeutic responses (Krstic and Knuesel 2012). Therefore, the development of technologies able to detect NDD-related lesions, particularly in the early steps of the disease before the onset of clinical symptoms and to monitor the progression of the disease, represents a great challenge. For this, the identification of reliable and specific biomarkers is required. Therefore, the quest for biosensing methods to follow up NDDs etiopathology non-invasively and cost-effectively is still ongoing.

In the area of medical nanotechnology, the development of more precise preparation methods at the nanoscale range in recent years has generated extensive research in the medical use of nanoparticles (NPs) for the delivery of curative agents. However, a little number of formulations were approved in the last years in spite of expansion of the number of clinical trials. On the other hand, it can be observed an important rise of other application of NPs in the medical field, that is, in vitro and in vivo diagnostic, imaging (Azria et al. 2017), biosensing (Howes et al. 2014; Shui et al. 2018), nonpharmacological therapeutic approaches (Palmal et al. 2014; John et al. 2018), and stem cell therapies (Santos et al. 2016).

The diverse usefulness of NPs is derived from their unique properties, such as their size in the 1–100 nm range, at the scale of most biological objects, their large surface to volume ratio generating large biological interfaces, and their possible functionalization with imaging or recognition ligands. Moreover, some nanomaterials have the ability to translate specific molecular interaction into specific signals which could be amplified to be finally detected by different modalities as discussed in the following sections.

A large range of nanomaterials (Fig. 2.1) from inorganic (metallic) to hybrid (organic/inorganic) and strictly organic NPs have been proposed for diverse CNS applications (Elsabahy et al. 2015; Yang et al. 2015; Smith and Gambhir 2017).

Inorganic particles represent a major part of those nanomaterials. Quantum dots (QD) are semiconductor nanocrystal (2–20 nm) with high brightness and narrow emission spectrum fluorescence properties. QDs are advantageous for in vivo applications (Zhou et al. 2015). Indeed, QDs allow to record images over a long period of time, thanks to their greater photostability than fluorescent dyes or proteins. Maysinger et al. (2007) visualized QDs up to 7 days post injection using in vivo imaging techniques. Mice were injected subcutaneously and scanned for fluorescence, in particular in the brain, where peak fluorescence was observable at 3 days post injection and persisted for 7 days (Maysinger et al. 2007).

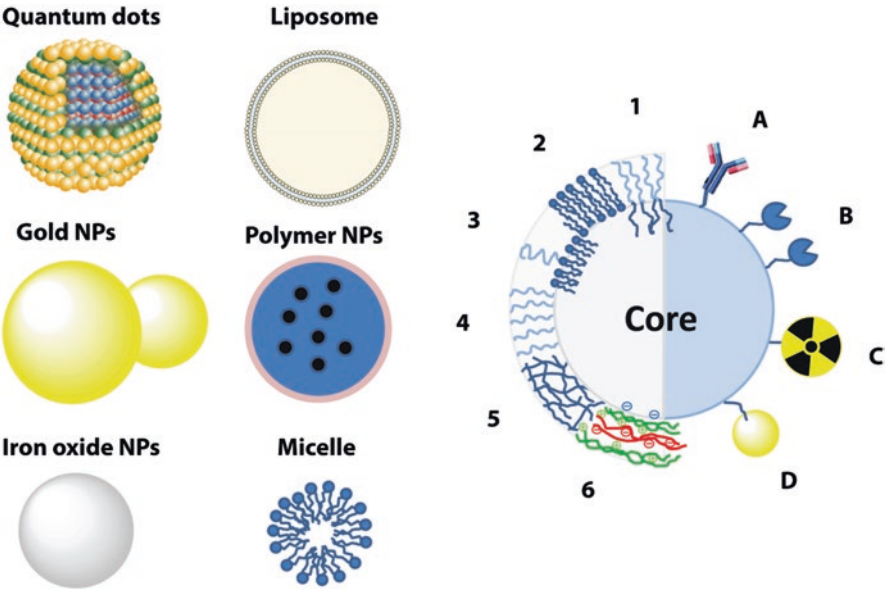

Fig. 2.1 Different types of particles involved in NDDs lesions diagnostic. On the left: Quantum dots (2–20 nm); gold nanoparticles (10–100 nm); iron oxide particles. Iron oxide particles include SPIO (superparamagnetic iron oxides, 60–120 nm), USPIO (ultrasmall SPIO, 20–50 nm), and MPIO (microparticle of iron oxide, 0.7–2 μm); organic particles: liposome (50 nm to 3 μm), micelle (5–100 nm), polymer nanoparticles (20–200 nm). Organic particles could be loaded with fluorescent dyes, semiconductor, or metallic particles for tracking and imaging. On the right: possible general surface modification of detection particles: (1) surface adsorbed polymers, (2) phospholipid bilayer, (3) phospholipid monolayer, (4) hydrophilic polymer layer tethered to the surface (e.g., Poly(ethylene glycol) chains added for stealthness and stability), (5) hydrogel layer, (6) layer-by-layer polyelectrolytes assembly; and specific modification with (A) specific targeting antibody or ligand, (B) specific recognition motif for molecules recruitment and detection, (C) radionuclide (such as ^{18}F and ^{11}C for PET imaging), (D) gold or iron oxide particle attachment (or inclusion inside the particle) for multimodal detection and imaging strategies (surface plasmon resonance, MRI, etc.). Drawings are not at scale. The given size intervals are an approximation

Metallic NPs of different shapes as gold nanoparticles (AuNPs) have attracted a great deal of attention as a sensitive technique for chemical and bioanalytical sensing and imaging in vitro and in vivo. AuNPs are biocompatible and display exceptional optical properties enabled by interaction of light with Au surface. AuNPs can change color upon analyst binding and NPs aggregation, displayed photoluminescent properties as well as surface plasmon properties enhancing Raman spectroscopy signals (SERS) (Yang et al. 2015).

Iron oxide NPs with magnetic properties are useful as contrast agent for magnetic resonance imaging (MRI). MRI is a powerful technique to image live tissue; however, it suffers from lack of contrast (Srikar et al. 2014; Sharifi et al. 2015). Different agents, including targeted metallic NPs (such as iron oxide particles in the 10–50 nm range), have been considered to enhance contrasted signals in brain MRI

(Daldrup-Link 2017). Paramagnetic ions such as Gadolinium (Gd) or ferric cations (Fe^{3+}) influence proton relaxation times around them, generating/enhancing contrast of magnetic resonance images. However, NPs considered in MRI detection of NDDs lesions must meet several criteria including low toxicity, translocation across the BBB, an optimal signal-to-noise ratio, and specificity toward protein deposits or potential biomarkers (Azria et al. 2017).

Different types of organic particles (lipid, polymer, and hybrid materials) are also described, often as vehicles for encapsulated functional molecule such as enzymes, reporter molecules, contrast agent, or fluorescent dyes (Niu et al. 2014). Fluorescent NPs possess several advantages over small fluorescent probes. NPs can have stronger fluorescent emission due to large number of fluorescent molecules encapsulated in each NP, while the high surface-area-over-volume ratio provides a higher probability for interactions and detection of the molecule of interest. The encapsulation of fluorescent probes protects them from external interference such as undesirable enzymatic reactions and nonspecific interactions with proteins.

All those NPs can be surface modified to enhance their stability in biological media (Rabanel et al. 2014). Surface functionalities such as targeted-specific ligand either for specific organ distribution and cell barrier translocation or for specific molecule detection can also be added to the nanodevice (Fig. 2.1).

NDDs diagnostic and monitoring could benefit from advances in NPs technologies. However, specific challenges are associated with the use of NPs in the CNS, mostly related to the organization of the organ and its vascular system (Wong et al. 2013; Lei et al. 2017) as well as the sensitivity of neural tissue to toxicity induced by nanomaterials (Oberdörster et al. 2009; Boyes et al. 2012).

The general anatomy of the brain features several distinctive structures. The most striking differences concern the vasculature with a tightly regulated permeability and particular waste management system evidenced by the absence of a regular lymphatic system. NPs penetration and distribution inside the brain are largely impeded by different brain barriers (Rabanel et al. 2012, Sweeney et al. 2019) and limitations of particles diffusion inside brain parenchyma (Thorne and Nicholson 2006; Pardridge 2016). Anatomy and brain physiology drastically limit distribution of NPs, be they are administered via the blood route, intracerebral injections, or other route (nasal and choroid plexus). However, pathological, anatomical, and physiological differences could account for reports showing detection enabled by NPs probes in brain parenchyma (Sweeney et al. 2019).

This chapter is devoted to the recently explored roles of NPs in physiology and physiopathology for in vivo investigations of the brain in the NDDs context. Vascular dysfunctions and change in brain parenchyma homeostasis is a common feature of all the NDDs. Detection and imaging of specific biomarkers for NDD by NPs, a key issue in clinical neurology, will be also presented. This chapter focuses on biomarkers effectively detected via nanoparticle-assisted technology. Readers interested in more technical issues regarding detection methods are referred throughout the chapter to recent reviews on different detection modalities.

2.2 Nanoparticles in Functional Investigation of Brain Vascular System Physiology and Physiopathology

The disruption of normal function of BBB has been linked to NDD (Zlokovic 2011; Sweeney et al. 2018). Vascular dysfunction plays a role in pathogenesis of AD (Di Marco et al. 2015). AD is accompanied by a decrease in microvessels density and cerebral global and regional blood flow (Kisler et al. 2017). Some evidences point out chronic cerebral hypoperfusion (CCH) as playing a role in AD progression. CCH is reported to cause reduced oxygen, glucose, and other nutrient supply to the brain, resulting in direct damage to the parenchyma, neurons, and the BBB integrity. The accumulation of amyloid-β peptide (Aβ) in AD could result from vascular dysfunction (such as impaired transport/elimination via BBB-specific transporter into circulation). At the same time, it could also mediate negative effects on the different cell types of the neurovascular unit contributing to the disease etiology (Kisler et al. 2017). Hypoperfusion has been also associated with PD (Heron et al. 2014), and BBB dysfunction has been identified in parkinsonian midbrain (Kortekaas et al. 2005). Additionally, leakage and microbleed, diminished glucose transport, and efflux pump dysfunction have been reported for several NDDs (Sweeney et al. 2018).

BBB's normal function disruption allows influx of toxic products into the brain and their accumulation due to imbalanced efflux processes. BBB disruption could also allow penetration of peripheral cells and pathogens. All these events lead to inflammation and immune responses, which can initiate or worsen several NDDs. So there is an interest in investigating different aspects of vascular physiology and physiopathology in NDD.

2.2.1 Testing the Integrity of the BBB with NPs: Leakage and Microbleed

Inflammation, tumor, or stroke can compromise the integrity of the BBB, at least on a temporary basis. Permeability dysfunctions could take different forms. The most relevant for NPs penetration are the modifications of the paracellular permeability and the modulation of transcytosis. The BBB integrity could be tested with nanotechnological tools. For instance, Yang et al. (2004) used 20-nm fluorescent-labeled polystyrene NP to study the BBB permeability following cerebral ischemia and reperfusion. NPs were injected in the cerebral cortex of rats, and transient accumulation of NPs in the extracellular space was observed by microdialysis probes (Chefer et al. 2009), following what could be a mechanism of extravasation from the blood vessels. The authors claimed that they have obtained high temporal resolution measurements of BBB permeability in vivo using this approach, correlating well with changes of cerebral oxygen concentration (Yang et al. 2004). Similarly, transient opening of the BBB was also evidenced by Smith et al. (2016) in a model of rat optical nerve injury. Upon

injury, BBB permeability change resulted in a dramatic change in the brain biodistribution of intravenously (i.v.) administered albumin-coated fluorescent NPs otherwise excluded from brain parenchyma (Smith et al. 2016).

2.2.2 Blood Flow, Volume, and Vascular Density

The cerebral blood flow and volume (CBF and CBV) can be indicative of pathological conditions or normal aging. CBF modulation has been linked to development of NDD (Zhang et al. 2017). CBF and CBV have been shown to precede cognitive decline in AD. CBF has been shown to decline in specific region associated with AD etiology (Kisler et al. 2017).

CBF and CBV can be investigated by magnetic resonance imaging (MRI). A critical issue for diagnostic and treatment is the quality of MRI images generated, and this is heavily dependent on the use of the proper contrast agent. It has been shown that the CBV mapping could help for diagnostic and stereotactic biopsy targeting (Varallyay et al. 2013). In this context, ultrasmall superparamagnetic iron oxide nanoparticles (USIONPs) Ferumoxytol®, an FDA-approved substance for iron replacement therapy, has been tested as a MRI contrast agent in the CNS. Because of its particle size (17–33 nm in diameter), Ferumoxytol® is retained in the blood compartment, with no significant leakage even if case of compromised BBB (Varallyay et al. 2013). In a study aimed at evaluating and comparing MRI techniques to detect alterations of brain microvasculature in AD animal models, Ielacqua et al. (2016) demonstrated that MRI was able to detect age-dependent changes of the cerebral vasculature in the arc Aβ mouse model of cerebral amyloidosis (CAA). Arc Aβ mice are transgenic mice overexpressing the human APP695. The analyses of relative CBV and relative CBF were performed by tracking super paramagnetic iron oxide NPs. Brain regional differences were observed for both parameters: the different mouse genotype and age groups. Microvessel density was independently carried out by vessel staining with CD31 and confirmed a reduction of microvessel density in the old (24 mouths) arc Aβ mice. This approach allows the detection of amyloid-related degeneration of the cerebral microvasculature (Ielacqua et al. 2016).

2.2.3 Cerebrospinal Fluid, Interstitial Fluid, and Lymphatic Drainage of the Brain

Cerebrospinal fluid (CSF) circulation provides a route for metabolism waste elimination. Disruption of normal CSF circulation and turnover may to contribute to the development of NDD including AD (Simon and Iliff 2016).

Carboxy quantum dot (QD) fluorescent NP (19 nm in diameter with an emission peak at 655 nm) was used to follow the CSF–lymphatic drainage of mice. The hyperspectral imaging allowed by QD revealed itself as a tool to study CSF–lym-

phatic drainage and could be relevant to understand this pathway in CNS disease models (Mathieu et al. 2013). In this study, negatively charged QDs were chosen because a negative surface charge has been shown previously to improve lymphatic uptake and retention. Organically modified silica (ORMOSIL) NPs encapsulated with IR-820 NIR fluorophores were synthesized and characterized. The NPs were used as near-infrared (NIR) fluorescent contrast agents for in vivo brain imaging of mice. The authors also showed their application to sentinel lymph node (SLN) mapping of mice (Qian et al. 2012).

2.2.4 Oxygen Level Detection

Oxygen level is an indicator of the metabolic state of an organ and may be related to cerebral blood flow. NPs can be conceived to monitor different physicochemical properties of the medium around them including oxygen level (Wang and Wolfbeis 2014). For instance, a class of small diameter core-shell NPs (<50 nm) possessing a biocompatible shell and a hydrophobic core with embedded oxygen-sensitive platinum-porphyrin (PtTFPP) dyes was developed by Liu et al. (2013a, b). The oxygen-sensing performance of the NPs was investigated in order to provide a NP oxygen sensor for precise mapping of the oxygen level of living cells and tissues (Liu et al. 2013a, b). A similar approach was proposed by Dimitriev et al. (2015).

2.2.5 Vascular Imaging Enhancement with NPs

Modulation of microvascular density in specific region of the brain is a marker of degeneration. Aberrant angiogenesis have been reported in PD and HD and reduced brain capillary density in AD and PD (Sweeney et al. 2018).Vascular imaging is a tool to assess these changes and is also benefiting from nanoparticular agents as contrast agents. Photoacoustic method was used to image rat brain vasculature using DSPE-PEG_{2000} matrix NPs associated with a NIR dye (with an absorption in the NIR range, 700–850 nm) as the contrast agent. This approach in spite of a limited resolution at this time provides a new way to image vasculature density (Liu et al. 2014).

2.3 Nanoparticles in Functional Investigation of Healthy, Neuroinflammation, and Neurodegeneration in Brain Parenchyma

Brain parenchyma is constituted by the ensemble of neural and glial cells, the extracellular matrix (EMC), and interstitial fluid (ISF), where neurotransmitters and other chemical signaling molecules are expressed, transported, and eliminated.

Development of noninvasive sensor is desirable to follow changes occurring during physiopathological conditions. During NDDs, brain parenchyma is the seat of different modification, from cerebral atrophy (Braak and Braak 1991), modulation of glucose and lactate (Zilberter and Zilberter 2017), as well as dissolved gases levels and neurotransmitters concentration alterations (Schliebs and Arendt 2011).

2.3.1 Brain Parenchyma, ECM, and Diffusion

The organization of the ECM varies during development and aging and is altered in NDDs. Alteration in ECM can have important pathological consequences for the brain, such as inhibition of axonal regeneration, impaired oligodendrocyte function, and axon remyelination (Lau et al. 2013).

Optical imaging shows that dextrans and QD with hydrodynamic diameters as large as 35-nm diffuse within the extracellular space (ECS) of adult rat neocortex in vivo. The modeling predicts a normal width of 38–64 nm markedly different value from earlier EM image analysis (Thorne and Nicholson 2006). Single-walled carbon nanotubes (SWCN) were followed by their near-infrared emission after their injection in rat cerebroventricles. They diffuse inside the ECS for tens of minutes in acute slices, and they can be used to directly observe these structures with high-resolution imaging. The authors were able to extract information about the dimensions and local viscosity of the ECM from the interplay of nanotube geometry and environment. Indeed, porosity and viscosity of ECS fluctuated locally and were found nonhomogeneous at the nanoscale (Godin et al. 2017).

2.3.2 Glucose Sensing

"Nanozymes," that is, synthetic molecules or NPs with catalytic activity, have been applied to glucose monitoring in the rat brain in real time. In a study by Cheng et al. (2016), hemin, a peroxidase mimetic, and glucose oxidase were combined inside a metal organic framework (MOF). The authors demonstrated that the proximity of the two enzyme systems allowed sensing of glucose with fast kinetics (Cheng et al. 2016). In this study, a microdialysis approach was used to follow the kinetic, and it is, thus, more a proof of concept than a complete in vivo sensing application at this time.

Raman spectroscopy reveals the molecular composition of a medium by exploiting the frequency shift of scattered light from molecules without the use of labeling (Fig. 2.2). The specific spectroscopic signature can be related to pathological conditions. Regular Raman spectroscopy generates weak signal which could be enhanced by different modalities including surface enhancement Raman spectroscopy (SERS) (Devitt et al. 2018). SERS can detect low level of specific molecules including neurotransmitters and proteins in biological media.

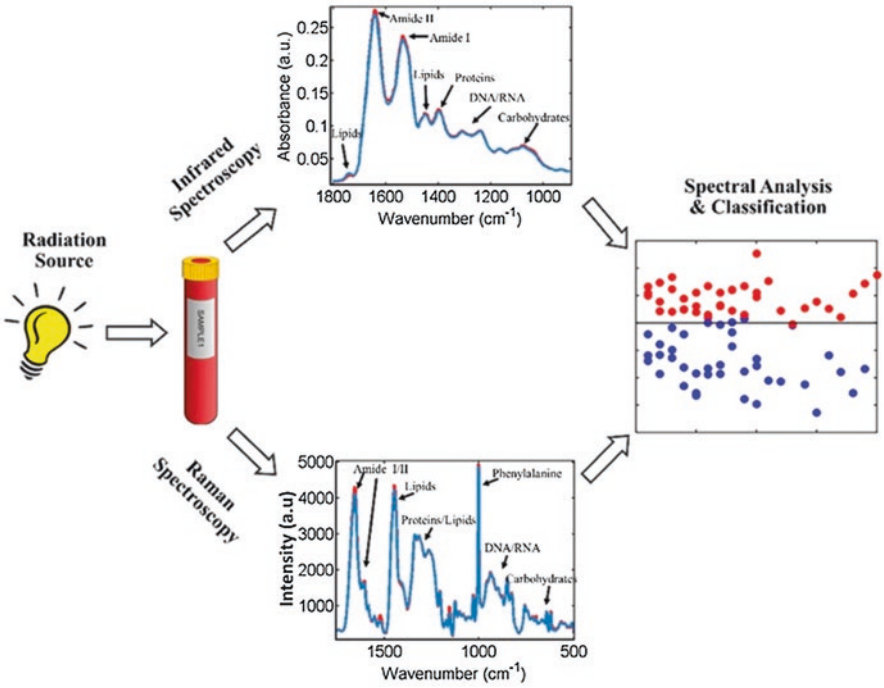

Fig. 2.2 Basic principle of infrared and Raman spectroscopy for biomarker analysis. A light source is directed to the sample to induce molecular vibrations. These vibrations are recorded, generating spectral information which is analyzed for biomarker identification. Reprinted and adapted with permission from Paraskevaidi, Martin-Hirsch et al. (2018); Copyright (2018) American Chemical Society

Gold NPs (AuNPs) possessing a peroxidase-mimicking nanozyme activity was designed to oxidize Raman-inactive reporter molecule leucomalachite green into the Raman-active malachite green (MG) with hydrogen peroxide. AuNPs were designed as the SERS substrates to enhance the Raman signals of the by-product of the reaction. Glucose oxidase and lactate oxidase enzyme were associated onto AuNPs for in vitro detection of glucose and lactate via SERS. The AuNPs or "nanozymes" were used to monitor the change of glucose and lactate in living rat brains, associated with ischemic stroke (Hu et al. 2017).

2.3.3 Functional Mapping of Brain Activity

2.3.3.1 Neurotransmitter

The changes in space and time of neurotransmitter secretion and concentrations are at the heart of cerebral normal or pathological functioning. Deficiency in neurotransmitter production and concentration is associated with several NDDs (Hardy

et al. 1985; Reinikainen et al. 1990; Xu et al. 2012). Therefore, the developments of biosensing to detect the levels of neurotransmitters in vivo and in real time can be viewed as a breakthrough tool in neuropharmacology to monitor neurodegeneration.

It had been proposed to use functionalized gold NP (AuNP) and QD surface modified with neurotransmitter receptors to provide a cellular-level resolution image of neural activities in real time. Optical properties (absorption/emission spectra) of AuNP and QD change upon binding (or removal) of ligand located at their surfaces. For instances, neurotransmitter (dopamine, gamma-aminobutyric acid (GABA), and glycine) binding to receptor immobilized on NP surface can result in signal shift detectable by different modalities (Forati et al. 2016). In this context, results demonstrated that QDs and gold nanorods with diameter around 25 nm and aspect ratios larger than three (ratio between length and width) were promising to record neurotransmitter binding signals and to develop functional brain mapping approach (Forati et al. 2016).The goal is to provide high spatial and temporal resolution maps of molecular events. Noteworthy at this point, it is only at the concept stage as the system is still to be validated on tissues and toxicity tests are still to be conducted.

The photostability of QDs allows the tracking of molecular event over a long period of time and with high resolution. The challenge is to translate these sensor systems from 2D culture systems (such as cell culture or acute tissue slide) to sensors able to detect the same molecular event in vivo (intact in vivo brain tissues).

CdSQDs (3.3 nm) were conceived with L-glutamic and L-aspartic as surface capping agents. The neurotransmitter moieties surface-modified QDs were developed to investigate cell targeting and signaling applications although they still had to be tested in vivo (Mansur et al. 2013). Another step toward clinical use has been made with the detection of serotonin and other neurotransmitters through cat skull by combining two types of SERS and spatially offset Raman spectroscopy (SORS), which provide enhanced signals generated by the presence of injected AuNPs. The reported serotonin detection limits are of 50 mM to 100 μM, too high at the present time for diagnostic or treatment follow-up. It could be expected a lowering of this detection limit with improvement of the technology in the future. This technique is, however, not providing neurotransmitter mapping of the brain (Moody et al. 2017).

2.3.3.2 Neurotransmitter Receptor Investigations

Altered expression of neurotransmitter receptor expression has been linked to susceptibility to several NDDs, such as HD, AD, ALS, and PD (Fu et al. 2018).

Antibodies-associated QDs were able to record lateral diffusion of neurotransmitter receptors at the surface of brain cells in vitro (Ciappelloni et al. 2017). QDs were tested to track single glutamatergic N-methyl-D-aspartate receptors (NMDAR) in acute brain slices. Functionalized QD (10–20 nm) was injected in cerebral ventricle to rats expressing tagged NMDAR, and the receptors were successfully

tracked in dissociated and native brain tissue. Ultimately, the goal is to investigate receptor diffusion biophysics in intact tissue and exploring the physiopathology roles of receptor surface dynamic (Varela et al. 2016b). The same team reported the tracking of dopamine receptor using the same approach (Varela et al. 2016a).

2.3.3.3 Other Functional Investigations

Amphetamines are known to affect neuroglia cells. Using fluorescent superparamagnetic iron oxide NP (SPION) surface modified with antisense oligodeoxynucleotide corresponding to glial fibrillary acidic protein (GFAP) mRNA, Liu et al. (2013a, b) were able to target neuroglia and recorded MRI images of SPION distribution in different settings. Living neural progenitor cells (PC-12.1), as well as the cells in fresh brain slices and intact mice brains, exhibited uptake of the antisense-modified SPION, but not SPION modified with scrambled and sense sequences. The presence of SPION was shown only in the neuroglia of normal or transgenic mice by TEM. Mice with acute and chronic amphetamine exposure displayed a reduction in striatal neuroglia. This study supports the feasibility of antisense-DNA targeting of SPION enabling in vivo MRI of neuroglia (Liu et al. 2013a, b).

Detection of epilepsy foci and their accurately mapping have been described using superparamagnetic NPs. The concept relies on the specific aggregation of NPs in the epileptic foci due to high electrical and magnetic activities. Pedram et al. (2015) present the mathematical models and simulation results showing the feasibility of such approach, improving the diagnostic and the subsequent treatment of this disease, by the improvement of the resection of epileptic foci (Pedram et al. 2015).

Detection of necrotic area following brain ischemia with optical probe consisting in PLGA-PEG NPs conjugated to 800CW molecular optical probe was tested in a mice model. Diffusion of NPs in traumatic brain injury has been found optimal for NP size of 100 nm, although smaller NP sizes were not tested. They appear not only to cross the BBB but also to diffuse in brain parenchyma (Cruz et al. 2016).

2.3.4 Neuroinflammation and Immune Cell Infiltration

NDDs are associated with the development of neuroinflammation. In MS, neuroinflammation is clearly characterized comprising infiltration of the brain parenchyma by peripheral lymphocytes and macrophages, impairment of BBB function, and strong microglia activation (Ransohoff 2016). Neuroinflammation is now recognized as an important contributor to AD and PD etiology. It is initiated via microglia activation by ROS, and Aβ production and inflammatory mediators are found in parenchyma along with activation of microglia and astrocytes (Van Eldik et al. 2016).

Ultrasmall paramagnetic iron oxide NPs (Ferumoxytol® or USPIO NPs) can enhance contrast between inflamed and normal brain tissue in MRI. Ferumoxytol NPs are surface modified by dextran and derivatives for colloidal stability issues in biological media. USPIO NPs were localized in brain tissue by using antidextran antibodies. When injected into rats with tumor-induced inflammatory brain lesions, it was found that USPIO NPs were taken up by astrocyte end-feets and macrophages, but not by tumor cells. This outcome suggests that USPIO NPs can be used to assess the inflammatory state of brain lesions (McConnell et al. 2016).

Vascular cell adhesion molecule (VCAM-1) is overexpressed at the BBB during neuroinflammation. Peptide directed to VCAM-1 was associated to long-circulating micelles loaded with MRI paramagnetic contrast agent Gd. MRI detectable micelles targeted toward VCAM-1 allowed an efficient visualization of brain inflamed regions in a mouse model of acute neuroinflammation (LPS-induced inflammation) (Garello et al. 2018).

Some CNS diseases are characterized by peripheral immune cells diapedesis across the BBB. Leukocyte infiltrations have been reported in MS and AD (Gerwien et al. 2016; Pietronigro et al. 2016; Sweeney et al. 2018). In this regard, peripheral immune cells tracking by MRI with iron oxide NPs provide a way to determine in space and time their involvement in neurological diseases and lesions, such as MS, ischemic stroke lesions, and tumors. Infiltration of macrophage could be an in vivo marker for diagnostic disease progression or for the determination of treatment efficacy (Petry et al. 2007). The ability of peripheral monocyte cells to cross BBB upon brain acute inflammation induced by LPS had been shown using monocyte loaded with SPIONs (Tong et al. 2016). Disruption of the BBB in ischemic stroke is followed by massive invasion of peripheral immune cells. Infiltration of T cells in a mouse experimental ischemic stroke was also reported using SPIO NPs conjugated to Rhodamine-B. The functionalized NPs were incubated with T cells from mouse spleen to get an effective labeling of the T-cell population following phagocytosis (Jin et al. 2016). Several examples of NP-assisted tracking of immune cells in MS are provided in Sect. 2.4.

2.3.5 Nanoprobes to Detect ROS and NOS Levels and Production

Imbalance of ROS and NOS levels is implicated in the regulation of cellular signaling cascade and has been proposed as an early event in several NDDs including AD (Markesbery 1997; Ramassamy et al. 2001; Barnham et al. 2004; Belkacemi and Ramassamy 2012; Kamat et al. 2016). Neurons are highly sensitive to oxidative stress that can induce apoptosis or necrosis. Moreover, the integrity of BBB can be compromised by oxidative stress, contributing to disease progression (Di Marco et al. 2015).

Detection method of peroxide has been reviewed recently including NPs as platform for ROS sensing (Uusitalo and Hempel 2012; Guo et al. 2014). For instance, peroxalate-based NPs emit fluorescent light upon excitation of an encapsulated dye on contact with H_2O_2. This approach was validated for in vivo imaging of H_2O_2 in a mouse model (Lee et al. 2007). Semiconducting polymer-based nanoprobe combining chemiluminescence imaging with ratiometric imaging allowed ROS sensing (ONOO and H_2O_2 detection) in the liver of living mice and in real time (Shuhendler et al. 2014). Single-walled carbon nanotube (SWNT) nanosensor exhibited high selectivity and sensitivity to single molecules of H_2O_2 (Kim et al. 2011). Fluorescent boronate-modified polyacrylonitrile NPs (50 nm in diameter) were shown to selectively detect H_2O_2 (Oh et al. 2012). However, all these approaches had not been tested in vivo. Furthermore, all these nanomaterials have still to be tested for their biocompatibility.

2.4 Nanoparticles for Functional Investigation of Alzheimer's Disease Markers

The definite diagnostic of AD is currently only available following *postmortem* neuropathological analysis. Available tests for suspected patients are invasive, available sparingly, or costly. There is still a need to develop diagnostic tools at the early stage of the disease in order to optimize available therapies, even before the mild cognitive impairment stage (MCI).

One of the hallmarks of the AD is the accumulation of Aβ into amyloid plaques in extracellular space producing a massive local neurodegeneration. Imaging of these amyloid plaques in live tissues is critical to perform diagnosis of AD, to follow the disease progression and treatment efficacy. Another AD-specific lesion is formed by the hyperphosphorylation of Tau protein, which causes its dissociation from microtubules and its aggregation in intracellular space into neurofibrillary tangles (Arriagada et al. 1992; Ingelsson et al. 2004). Therefore, there is tremendous interest in developing molecular imaging agent which can detect AD lesions based on these pathological hallmarks (Tables 2.1 and 2.2). These agents have to be nontoxic and ideally be able to cross the BBB without facilitation.

2.4.1 Detection and Imaging of Amyloid Plaques

Detection and imaging of amyloid plaques rely on three elements: (1) A detection tools (USPIOs, MNPs, etc.) able to generate a signal, (2) a recognition element for the specificity (Aβ peptide, RNA aptamer, antibody, etc.), and finally, (3) a way to cross the BBB (with or without the BBB opening).

Table 2.1 Nanoparticle-assisted brain physiopathology investigation: selected examples

Disease marker	Nanoparticle	BBB crossing	Detection modality	In vitro /In vivo	Reference
Vascular marker					
Vascular imaging	DSPE-PEG2000 NPs	No	Photoacoustic NIR	In vivo (rat)	Liu et al. (2014)
Blood–brain barrier integrity	Polystyrene NPs	Yes	Fluorescence	In vivo (rat)	Yang et al. (2004)
	Albumin-coated NPs	Yes (upon injury)	Fluorescence	In vivo (rat)	Smith et al. (2016)
Cerebral blood flow and volume	USPIO NPs	No	MRI	In vivo (human)	Varallyay et al. (2013)
Lymphatic drainage	QD	No	Fluorescence	In vivo (mouse)	Mathieu et al. (2013)
Oxygen detection	Platium-porphyrin-polystyrene NPs	No	Phosphorescence emission	In vitro	Liu (2013), Dmitriev et al. (2015)
Brain parenchyma					
Diffusion in ECM	QD	NA	Fluorescence	In vivo/open cranial window (rat)	Thorne and Nicholson (2006)
	Single-wall carbon nanotube	No (injection icv)		In vivo (rat)	Godin et al. (2017)
Glucose sensing	AuNPs Nanozymes	Yes	Raman (SERS)	In vivo/ microdialysis (rat)	Cheng et al. (2016), Hu et al. (2017)
Neurotransmitter	Antibody-modified QD and AuNPs	NA	Fluorescence shift	In vitro	Forati et al. (2016)
	Serotonin	NA	SERS	Brain tissue mimic	Moody et al. (2017)
Necrotic area	PLGA-PEG NPs (100 nm)	Yes (I.V)	NRI fluorescence	In vivo (mouse)	Cruz et al. (2016)
Inflammation	USPIO NPs	No	MRI	In vivo (rat)	McConnell et al. (2016)
	VCAM-1 targeted paramagnetic micelle	No	MRI	In vivo (mouse)	Garello et al. (2018)
Immune cell infiltration	Iron oxide NPs in monocytes	Yes	Time lapse MRI	In vivo (mouse)	Masthoff et al. (2018)

DSPE-PEG2000 Distearoyl-sn-glycero-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000, *NRI* near infrared, *USPIO NPs* ultrasmall paramagnetic iron oxide NPs, *MRI* magnetic resonance imaging, *QD* quantum dot, *ECM* extracellular matrix, *AuNPs* gold nanoparticle, *SERS* surface-enhanced Raman spectroscopy, *NA* not applicable

Table 2.2 Alzheimer's disease: selected examples of nanoparticle-assisted disease marker detection

Disease marker	Nanoparticle	BBB crossing	Detection	In vitro/ In vivo	Reference
Amyloid plaques	MNP-antiferritin	Facilitated (mannitol)	Immunofluorescence	In vitro	Fernández et al. (2018)
			MRI	Ex vivo	
	USPIO-PHO	Without facilitation	MRI	In vivo	Ansciaux et al. (2015)
	USPIO-PEG-Aβ (1-42)		mMRI	In vivo	Wadghiri et al. (2013)
				Ex vivo	
	Liposomes-ET6-21		NIR	Ex vivo	Tanifum et al. (2016)
			Immunohistochemistry		
			MRI	In vivo	
	Cur-MNP		Immunohistochemistry	In vitro	Cheng et al. (2015)
	NPs-BSA-Sia		Fluorescence	In vitro	Nasr et al. (2018)
			MRI	In vivo	
Cerebrovascular amyloid deposit	MION	No need of BBB crossing (vessel's wall)	MRI	Ex vivo	Poduslo et al. (2011)
	Cross-linked chitosan	Antibody targeted to vessel wall	MRI/SPECT	In vivo	Jaruszewski et al. (2014)
Tau tangles	AuNP-anti-tau	No need of BBB crossing (CSF)	TPRS	In vitro	Neely et al. (2009)

MNP magnetic iron oxide nanoparticles, *MRI* magnetic resonance imaging, *μMRI* magnetic resonance microimaging, *NRI* near infrared, *USPIO* ultrasmall particles of iron oxide, *MION* monocrystalline iron oxide nanoparticles, *SPECT* single-photon emission computed tomography, *AuNPs* gold NPs, *TPRS* two-photon Rayleigh scattering

2.4.1.1 Strategies with Facilitation of BBB Crossing

Temporary disruption of the BBB is usually achieved by intracarotid infusions of hyperosmotic solutions such as mannitol. Mannitol has been the most widely investigated agent, causing reversible shrinkage of brain ECs by water efflux, leading to tight junctions (TJ) disruption and increased drug diffusion to the brain (Kroll and

Neuwelt 1998). This strategy was also proposed to enhance brain delivery of NPs (Muldoon et al. 1995).

Magnetic NPs (Fe_3O_4-dextran-coated NPs) bound to an antiferritin antibody allow targeting of the amyloid plaques because of increased level of the ferritin protein in areas with high accumulation of amyloid plaques (particularly the subiculum in hippocampal area). MNP-antiferritin conjugates were detected in vitro by immunofluorescence in brain slides. They were also tested in vivo by intravenous injection in AD model mice (5xFAD) where the BBB crossing was facilitated by administration of mannitol. Six hours after the intravenous administration, the MNP-antiferritin conjugates were visualized by immunofluorescence and by ex vivo brain MRI (Fernández et al. 2018). However, the risk of toxic side effects limits this strategy to highly aggressive and life-threatening diseases (Cupaioli et al. 2014). Research more recently focuses on the development of strategies without BBB disruption.

2.4.1.2 Strategies without Facilitation of BBB Crossing

The ultrasmall particles of iron oxide (USPIOs) functionalized with peptides presenting a nanomolar affinity for Aβ (USPIO-PHO) were described by Ansciaux et al. (2015). Biodistribution studies demonstrated that USPIO-PHO was able to cross the BBB without any facilitating strategy and accumulate in the brain 90 minutes after its injection in mice. Amyloid plaques were efficaciously labeled in the brain. NPs displayed no toxic effects and were characterized by an elimination half-life of about 3 h (Ansciaux et al. 2015).

Several approaches take advantage of the affinity of exogenous injected Aβ (1-42) peptides to target amyloid plaques (Gureviciene et al. 2017). Therefore, several workers proposed to use this property of the Aβ peptide to colocalize imaging NPs with plaques. USPIOs were chemically coupled with Aβ (1-42) to target amyloid plaque and PEG chains to improve BBB permeability. These USPIOs were injected i.v. in the transgenic APP/PS1 mice model of AD and followed by in vivo and ex vivo magnetic resonance microimaging (mMRI). The amyloid plaques detection by mMRI was confirmed with matched histological sections. Comparison of USPIO-PEG-Aβ1-42 and USPIO alone injected in AD transgenic mice demonstrated that USPIO-PEG-Aβ1-42 can be used for amyloid plaque detection in vivo without the need to co-inject a BBB permeabilizing agent (Wadghiri et al. 2013). The addition of PEG chains on the surface of USPIO has for consequence a longer circulation time, increasing the BBB crossing.

Koffie et al. (2011) tested a nanoparticular system made up of poly(n-butyl cyanoacrylate) dextran polymers coated with polysorbate 80 (PBCA NPs) to deliver BBB-impermeable molecular imaging probes into the brain for targeted imaging. The authors reported that PBCA NPs allowed rapid delivery of an enhanced dose of BBB-impermeable molecular probes. The cargo tested were molecules such as Texas red and Trypan blue, two dye binding amyloid plaques as well as anti-Aβ antibody and MRI gadolinium-based contrast agent for brain imaging. PBCA NPs

translocate across the BBB apparently using an ApoE-dependent mechanism. This delivery approach utility was demonstrated by visualization of amyloid plaques in vivo in a mouse model of AD (Koffie et al. 2011). Noteworthy about 5% of the dose is brain delivered, which is sufficient for imaging; nevertheless, about 95% of the dose is found elsewhere in the body.

A new Aβ-binding molecule (ET6-21) with greater hydrophilicity than existing ligands was synthesized by Tanifum et al. (2016). The ligand was used to prepare novel amyloid-targeted liposomes tagged with MIR and NIR probes. I.v. injected liposomes-ET6-21 were successfully translocated across the BBB and were able to bind to the amyloid plaques in transgenic mice brains. MRI demonstrated the presence of probe signal in the brains of mice with amyloid plaques, while no signals were observed with an untargeted version of the liposome (Tanifum et al. 2016).

The polyphenol antioxidant, curcumin, has been also proposed to detect senile plaques. Indeed, curcumin has binding properties for amyloid deposits. Superparamagnetic iron oxide (SPIO) particles surface modified with curcumin were further modified with PEG-PLA and PVP polymer, forming stable 100-nm curcumin magnetic NPs (Cur-MNPs). Cur-MNPs were shown to allow the visualization of amyloid plaques in ex vivo T2∗-weighted MRI of genetic AD mice (Tg2576) brains. No plaques could be detected in control mice. Examination of the mouse brains by immunohistochemistry revealed that Cur-MNPs were co-localized with amyloid plaques (Cheng et al. 2015).

Bovine serum albumin (BSA)-coated iron oxide NPs decorated with sialic acid (NP-BSA-Sia) were found to cross a BBB in vitro model. MRI showed NP-BSA-Sia high selective binding to amyloid plaques in human AD transgenic mouse brains. Noteworthy, NPs can be detected without having to artificially increase BBB permeability (Nasr et al. 2018).

2.4.2 Detection of Cerebral Angiopathy Amyloid

Cerebral angiopathy amyloid (CAA) is the deposit of Aβ peptide inside the vascular wall of small arteries of the cerebral cortex and leptomeningeal blood vessels (cerebrovascular amyloid, CVA). It is found associated with AD but can also be found in healthy older patients. CAA increases vessel fragility, risk of hemorrhage, and risk of dementia. The development of CAA-like pathologies has been observed in clinical trials testing Aβ antibody treatments for AD (Banerjee et al. 2017).

Poduslo et al. (2011) studied the detection of CVA by MRI using immunotargeted monocrystalline iron oxide NPs (MIONs). Ex vivo, MIONs were detected specifically bound to the amyloid deposits in the brain from the AD transgenic mice Tg2576. In the case of CAA, NPs do not have to cross the BBB and can directly interact with vessel CVA (Poduslo et al. 2011). A monoclonal antibody raised against human fibrillar Aβ42 was conjugated to NP prepared by ionic gelation and chemically cross-linked. These NPs were loaded with gadolinium, MRI contrast agents, or single-photon emission computed tomography (SPECT) agents. NPs

were able to target CVA and to serve as early diagnostic agents in a model of APP mice (Jaruszewski et al. 2014).

2.4.3 Detection of Tau Phosphorylation and Aggregation

Alternative therapeutic target to Aβ has to be developed for AD treatment because there is no clear correlation between Aβ plaque levels and cognitive decline (Arriagada et al. 1992; Ingelsson et al. 2004). Moreover, several strategies to target Aβ deposit have recently failed in separate Phase III clinical trials (van Dyck 2018). These results may suggest that Aβ plaques are a consequence rather than a cause of cognitive decline.

Another hallmark of AD is protein Tau hyperphosphorylation and resulting intracellular tangle formations (Iqbal et al. 2015). Tau protein is involved in stabilization of microtubules and, thus, plays an important role in the neuronal structure and function. Hyperphosphorylation of Tau leads to microtubules destabilization. Moreover, hyperphosphorylated proteins aggregate to twisted insoluble fibers, the intracellular neurofibrillar tangles which are correlated with cognitive decline (Arriagada et al. 1992; Berg et al. 1998).

Hyperphosphorylated Tau can be found in CSF and plasma of AD patients and its levels correlated with the disease progression (Chiu et al. 2014). Neely et al. (2009) demonstrated the detection of hyperphosphorylated Tau protein in CSF by anti-Tau antibody-coated gold nanoparticles (AuNP-anti-Tau) observed by two-photon Rayleigh scattering spectroscopy (TPRS) (Neely et al. 2009).

For in vivo application, nanocomposite particle combining anti-tau aggregation properties and hyperphosphorylated tau recognition were constructed. The nanocomposite associated iron oxide and ceria ultra-small particles anchored on amine-modified mesoporous silica NPs. They were further modified with a phosphor-Tau ligand and silica pores filled with methylene blue a molecule know to prevent Tau aggregation (Chen et al. 2018). The resulting NP (100 nm) has bimodal imaging capabilities by MRI and PET, while they possess targeting properties as well as multiple therapeutic effect. They can scavenge ROS and prevent Tau hyperphosphorylation by the mean of immobilized ceria NP and prevent Tau aggregation thanks to the release of methylene blue. These effects have been shown in vitro and in vivo upon NP injection (Chen et al. 2018). The question of whether these multifunctional particles could effectively cross the BBB remains to be determined.

2.5 Nanoparticles in Functional Investigation of Parkinson's Disease and Other NDDs

NP-mediated biomarker detections have been also reported of PD and several other NDDs (Table 2.3). PD is associated with the selective loss of dopamine (DA) neurons in the *substantia nigra* and a decreased level of DA in the striatum. This loss of DA

Table 2.3 Parkinson and other diseases: selected example of nanoparticles detection of specific markers

Disease marker	Nanoparticle	BBB crossing	Detection modality	In vitro/In vivo	Reference
Parkinson					
Dopamine	PEGylated PFPBA NPs (120 nm)	Yes	Fluorescence quenching	In vivo (zebrafish)	Qian et al. (2015)
	PEGylated PFPBA NPs (100 nm)	Yes	NIR fluorescence	In vivo (mouse)	Feng et al. (2018)
Dopamine receptor	Immuno-targeted far-red QDs	Intraventricular injection	Fluorescence	In vivo (rat acute brain slide)	Varela et al. (2016a, 2016b)
α-Synclein	Gold nanorod	NA	Surface plasmon	In vitro	Kumar et al. (2018)
	Immuno/ magnetic particle	NA	Immunoassay	In vitro	Yang et al. (2016)
Multiple sclerosis					
Vascular inflammation	Anti-ICAM-1 MPIO	Yes	MRI	In vivo (mouse model of MS)	Blezer et al. (2015)
	Anti-VCAM-1 MPIO	Yes	MRI	In vivo (mouse model of MS)	McAteer et al. (2007)
Immune cells activation	Iron oxide NPs	Yes	MRI	In vivo (mouse model of MS)	Kirschbaum et al. (2016)
Peripheral immune cell infiltration	Iron oxide NPs in T cells	Yes	MRI	In vivo (mouse)	D'Elios et al. (2018)
	Iron oxide NPs in T cells	Yes	MRI	In vivo (mouse)	Anderson et al. (2004)
Huntington disease					
Oligomer amyloid protein	Iron oxide NPs	Yes	MRI	In vivo (mouse)	Liu et al. (2019)

NIR near infrared, *MPIO* magnetic particle iron oxide, *MRI* magnetic resonance imaging, *NA* not applicable

leads to motor symptoms (resting tremor, rigidity, etc.) as well as other symptoms including cognitive deficit (Rodriguez-Oroz et al. 2009). PD, the second most frequent NDD, is also characterized by amyloid accumulation of α-synuclein (Goedert 2015).

2.5.1 Dopamine and Dopamine Receptor Detection

In Sects. 2.3.1 and 2.3.2, several studies depicted the feasibility of dopamine level monitoring of dopamine receptor investigation using nanotools. Dysregulations of the dopaminergic system are associated with PD. Altered diffusion of surface dopamine receptors has been linked to modulation of synaptic plasticity. Targeting dopamine receptors in vivo with functionalized QD allows their tracking in acute rat brain slices (Varela et al. 2016a).

Neurotransmitter dopamine brain level is correlated to different conditions, including depression, PD, and drug addiction. In the zebrafish, an in vivo light transparent model, the detection of dopamine was achieved with fluorescence sensing NPs microinjected into the larvae brain ventricle (Qian et al. 2015). NIR nanoprobes allow imaging of neural activity linked to dopamine production in midbrain of the normal and addicted mice (Feng et al. 2018).

2.5.2 α-Synuclein Detection in PD

PD is associated with amyloid deposit of α-synuclein intracellularly forming oligomeric species then aggregating into fibril upon phosphorylation. Oligomers are considered as neurotoxic. Oligomer levels have been detected in the CSF and plasma of PD patients. Synuclein is a plasma marker of cognitive decline, but not a marker of the decline in motor activity in PD (Lin et al. 2017). Several NP-assisted detection methods from biological fluid have been devised. For instance, Kumar et al. (2018) demonstrated that gold nanorods can interact specifically with fibrillar form of α-synuclein via noncovalent interactions, generating surface plasmon resonance signals in vitro (Kumar et al. 2018). CSF provides a reliable detection of synuclein; however, the sample collection is invasive. On the other hand, plasma level of synuclein is below the detection limit of most technique. Yang et al. (2016) proposed to combine immunoassay with magnetic particle to distinct PD from PD with dementia using plasma samples which are much easier to collect (Yang et al. 2016).

2.5.3 Multiple Sclerosis

Multiple sclerosis (MS) is characterized by the formation of inflammatory lesions mainly in the brain and spinal cord white matter. These lesions are characterized by neuronal and axon loss and axon demyelination. MS is also characterized by microglial cell activation as well as migration of peripheral macrophages across a compromised BBB mediating demyelination and axonal damage (Petry et al. 2007). Leukocyte infiltration is possible due to the activation of vascular endothelial cells, enhancing expression of adhesion molecules such as VCAM-1 and intercellular cell adhesion molecule-1 (ICAM-1).

Autoimmune encephalomyelitis (EAE) is a mouse model of MS. One of the early events in EAE etiology is upregulation of ICAM-1 on endothelial vascular cells. ICAM-1 could be then used as biomarker of disease progression and used to validate therapies. The accumulation of iron oxide micron-sized particles (MPIO) surface modified with anti-ICAM-1 antibody was recorded by MRI. EAE animals injected with targeted MPIO displayed MRI hypointensities particularly in the subarachnoid space. ICAM-MPIOs were associated with cells located at the luminal side of blood vessels. Untargeted MPIO accumulation did not differ between the phases of EAE and was not associated with BBB dysfunction ICAM-1 (Blezer et al. 2015). Similarly, MPIO was used to detect VCAM-1 in vivo in mouse model of acute brain inflammation (McAteer et al. 2007).

Migration of T cells has been observed by labeling naive T cells after their exposition to myelin peptide with MRI contrast agent. Upon exposition to myelin peptide, these cells become able to induce relapsing-remitting EAE in receiving mice. T cells were labeled in vitro with SPIO NPs (Ferumoxides) complexed to poly-L-Lysine (Fe-PLL). After EAE onset, Fe-PLL–labeled T cells were detected in the mouse spinal cord using in vivo and ex vivo MRI. MRI signals colocalized with spinal cord lesions were assessed by histopathology (Anderson et al. 2004).

Monocytes loaded with iron oxide NPs were used for time-lapse MRI experiments. They were injected into EAE-bearing mice. Imaging provided by iron oxide NPs was able to provide insights into immune cell dynamics in mouse brain at a single-cell level in vivo (Masthoff et al. 2018). Myelin-specific T cells were efficiently loaded with polymer NPs with a magnetite core (NBR) and injected i.v. into C57Bl/6 naive or EAE-bearing mice (NBR loaded $CD4^{+}$T lymphocytes, autoreactive for myelin antigens). T cells loaded with NBR are able to migrate in the brain, where they were found in demyelinating lesions by immunohistochemistry of brain and spinal cord tissues (D'Elios et al. 2018).

MRI was also used to map brain resident and infiltrated innate immune cells in MS disease model using iron oxide NPs and visualize them in inflammatory lesions. It was shown that NPs uptake in EAE mice (MRI signal quantify phagocytic activity) colocalized with infiltrating macrophages and resident microglia markers and correlate with clinical disease severity (Kirschbaum et al. 2016).

2.5.4 Huntington's Disease

NDDs, including PD, AD, and Huntington's disease (HD), are all associated with protein aggregation. Misfolded amyloidogenic proteins, such as α-synuclein, mutant Huntingtin (mHTT), and Aβ, can aggregate into soluble oligomers, protofibrils, and insoluble fibrils. Despite the differences in amino acid sequences, amyloid proteins share common structural features allowing conformation-dependent, oligomer-specific antibodies to recognize diverse amyloid deposits. Liu et al. (2019) conjugated an amyloid oligomer-specific scFv antibody (W20) to PEGylated SPIONPs. W20 specifically recognized amyloid oligomers but not monomers and fibrils.

W20-SPIONPs, when injected to transgenic mice models of PD and HD, were able to cross the BBB and specifically bound to oligomers to give distinct MRI signals for transgenic versus healthy controls (Liu et al. 2019).

2.6 Perspectives and Outlook

The use of NP as diagnostic tools for NDDs is well beyond speculation as testable materials are already described. However, the efficacy of in vivo imaging and diagnostic approaches are currently hindered by several issues. The first issue is related to NP penetration and distribution into brain tissues (Fig. 2.3). The second one is the acute and long-term toxicity of NPs, in particular, the toxicity linked to the sensitivity of CNS to oxidative stress and thus to metallic functional NPs (Cupaioli et al. 2014; Bencsik et al. 2018). Lastly, a high signal-to-noise ratio and sensitivity to physiological and clinical relevant concentration are needed for an efficient detection in vivo.

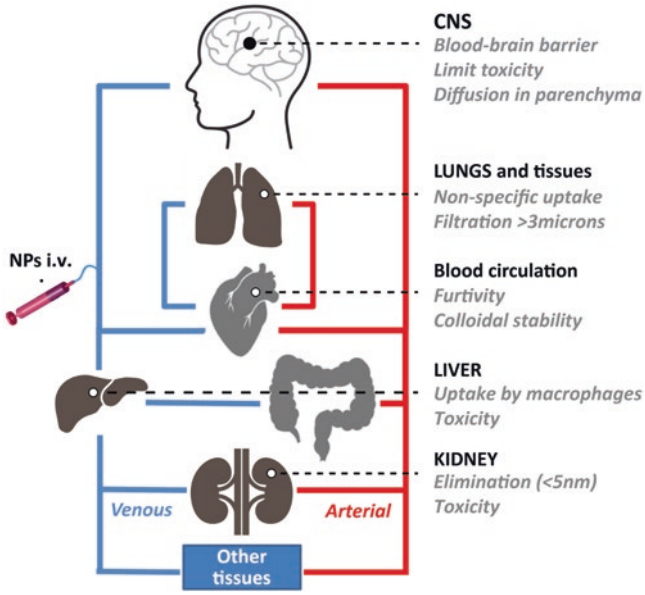

Fig. 2.3 Mean challenges associated with in vivo nanoparticle-assisted biomarkers detection (biodistribution aspects). Detection/imaging NPs i.v. administered are immediately opsonized in blood and could be recognized and eliminated by macrophage. NPs can be nonspecifically adsorbed on different biological interfaces including lungs. NPs can be subjected to elimination by organs such as liver and kidney according to their physicochemical properties. Likewise, NPs can be accumulated in CNS tissues in accordance to their physicochemical properties or their ligand-functionalized surface. The challenges associated with the use of NPs for NDD lesions detection are (1) the blood–brain barrier crossing, (2) the diffusion in parenchyma, and (3) the limitations of toxic effects on brain-sensitive cells (such as reactive oxygen species generation by metallic NPs)

In the last few years, the major trends are linked with identification of new type of nanomaterials with enhanced optical and detection capabilities; a trend toward more biomimetic nanomaterials to take advantage of properties of natural circulating NPs and finally the combination of different functions on the same particle, that is, theranostic NPs or NPs combining different detection modalities.

Biomimetism consists of surface-modified NPs with cell surface features in order to fulfill several objectives such as stealthness for a longer residence in blood circulation (Hu et al. 2013) or for specific targeting (Gong and Winnik 2012). For instance, natural occurring NPs have been inspiration to construct lipoproteins and lipoprotein mimetics for imaging (Cormode et al. 2014). Artificial HDL or LDL can be prepared and associated with an imaging probe to explore their role in brain (Thaxton et al. 2016).

Nanomaterials combining multiple functions are another new trend, and several concepts have been put forward in the last few years. For instance, in NDD application, a prototypical example is Congo red/Rutin magnetic NPs designed to specifically detect amyloid plaques by MRI, realize targeted delivery, achieve drug-controlled release by H_2O_2 response, and prevent oxidative stress. In vitro these NPs were shown to reduce the production of NO and ROS. Following i.v. administration resulted in detection of amyloid plaques and rescuing memory deficits in double-mutant transgenic mice APPswe/PS1dE9 (Hu et al. 2015). Image-guided therapy NPs have been described with plasmonic-magnetic NPs. The NPs are constituted of a magnetic core NPs surface modified with a gold shell. NPs were further modified with a PEG layer. These NPs are able to generate MRI contrast signal due to their magnetic properties, while gold surface can generate X-ray computed tomography (CT) signature. They show excellent biocompatibility and translocation across BBB model in vitro (Tomitaka et al. 2017).

Diverse physiopathology targets and disease markers could be now efficaciously detected with the assistance of these technologies. However, the development of in vivo nanoparticle-assisted diagnostic tools for NDDs is dependent on conception of nanomaterial taking in consideration the constraints of the brain anatomy and brain physiopathology (Wong et al. 2013). Brain barriers crossing and diffusion of NP in CNS tissues are necessary for most applications. One conclusion that can be drawn from the literature is that more information about fluid movements, active transcellular transport, and organ compartmentalization is needed in order to implement these new diagnostic and therapeutic approaches.

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Chapter 3
The Role of Nanomedicine in the Treatment of Neurodegenerative Disorders

Syed Tazib Rahaman

Abstract Neurodegenerative disorders are responsible for malfunctioning of brain and peripheral nervous system. The therapeutic drugs that are commonly utilized for the treatment of these disorders are not able to pass through the blood–brain barrier (BBB) as it permits passage of specific nutrients which are helpful for growth. Thus, nanotechnology (NT) can be very much valuable in solving this problem as there are different forms of nanomaterials that can act as efficient drug delivery systems and help in crossing the BBB and providing effective treatment for neurodegenerative disorders like Alzheimer's disease, Parkinson's disease, and many more. Thus, in this chapter, we tried to conglomerate all the recent developments related to neurodegenerative disorders and also provide a brief overview about the importance of nanotechnology in varied biomedical applications.

Keywords Neurodegenerative disease · Nanoparticles · Blood–brain barrier · Nanotechnology

3.1 Introduction

3.1.1 Neurodegenerative Diseases

Neurodegenerative diseases (ND) are heterogeneous type of disorders that are caused due to malfunctioning of brain or peripheral nervous system. They are caused due to malfunctioning of brain or peripheral nervous system. The term ND explains a varied range of conditions that majorly affect the neurons in human brain.

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Neurons are said to be considered as building blocks of the nervous system including the brain and spinal cord. Neurons do not have the capacity to reproduce or replace themselves. Age factor also plays a major role in neurodegenerative disorders. Alzheimer's disease (AD) and Parkinson's disease (PD) are most frequently caused disorders of this heinous type of disease. The combinational reaction of gene with the surroundings also leads to these disorders according to recent scientific reports.

There are many environmental factors, such as pesticides, metals, chemicals, and biological factors such as endotoxins, which cause ND.

3.1.2 Nanoscience

Nanoscience is a rapidly growing field of science which involves the study of structures and materials in the nanometer scale. Thus, nanoscience involves manipulation of atoms and molecules at supramolecular scale. Specialized methodologies are used to manufacture objects in nanoscale. There are various type of nanosubstances fabricated, such as nanofibres, nanocrystals, and quantum dots. Nanoelectromechanical systems (NEMS) are those devices which are used to carry out tasks which are too small for humans to do by themselves. High-powered microscopes are used to magnify nanoparticles. Scanning electron microscopes, nanoindenters, electrospinning equipment, optical profilers, and atomic force microscopes are generally used for the characterization of nanoparticles. There are varied types of metallic and biosynthesized nanoparticles (NPs) which are prepared through physical, chemical, and biological methods.

3.1.3 Nanobiomaterials

Biomaterials are biological substances which are introduced into the body as part of a medical device which replaces an organ or body function. According to their bioactivity, biomaterials can be classified into bioinert, alumina dental implant, bioactive hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] which are coated on a metallic dental implant, surface active bioglass, and bioresorbable tricalcium phosphate [$(Ca_3(PO_4)_2$] implant. Bioinert biomolecules have very less interaction with the surrounding tissue, whereas bioactive biomolecules undergo greater interaction with surrounding tissues including the softer ones. There is a lot of scope of manufacturing nanoparticles from biomaterials. Thus, according to a study done by Han et al. (2006), silver nanoparticles can be produced by following green synthesis in which reduction of silver nitrate occurs in the presence of water-soluble polymer such as poly-L-lysine. This study brings water-soluble polymers into limelight as an effective biomaterial in the production of varied type of nanoparticles. With the help of nanoparticles, we can also test the efficiency of varied biomaterials. The study by Attia et al. (2005)

supports the above statement, in which hyaluronic acid (HA) was produced by adding amino acid, which are said to be bioadditives, and was also prepared in the presence of magnetic nanoparticles, and the results were compared. In the presence of bioadditives, it showed highest dry weight of HA by adding glutamic acid (GA), whereas the preparation done in the presence of magnetic NPs showed highest dry weight of HA after addition of Fe_3O_4NPs which helped in evaluating the efficiency of NPs in the preparation of HA.

3.1.4 Polymeric Micelles and Other Nanoparticles

Polymeric micelles ("micellar nanocontainers") were developed as carriers of drugs and diagnostic imaging agents. They were formed spontaneously in aqueous solutions of amphiphilic block of copolymers and had a core-shell architecture along with a core of hydrophobic polymer blocks (e.g., poly(propylene glycol) (PPG), poly (D,L-lactide), poly (caprolactone)) and a shell of hydrophilic polymer blocks (often PEG). The size of polymeric micelles usually varies from ca. 10 to 100 nm. Their core can be incorporated into considerable amounts (up to 20–30% wt) of water-insoluble drugs which prevented premature drug release and degradation.

The shell stabilized various micelles in dispersion and masked the drug from interactions with serum proteins and untargeted cells. After they reach the target cells, drug was released from the micelle via diffusion. Several clinical trials were completed or underway, which were used to evaluate polymeric micelles for delivering anticancer drugs. One of the early studies used micelles of Pluronic® block copolymers (PEG-b-PPG-b-PEG) as carriers for CNS drug delivery (Duncan 2003).

These micelles were conjugated with either polyclonal antibodies against brain α2-glycoprotein or insulin as targeting moieties. Both antibody- and insulin-vectorized micelles were shown to deliver a drug or a fluorescent probe to brain in vivo. Furthermore, there was a considerable increase in neuroleptic activity of a drug (haloperidol) which was solubilized in the targeted micelles compared to a free drug. Subsequent studies demonstrated that the insulin-modified micelles undergo receptor-mediated transcytosis in BMVEC from luminal (blood) to abluminal (brain) side (Woods 2003).

Polymer NPs are said to be a better type of nanocarriers as they have greater biocompatibility and biodegradability than others. These NPs are synthesized from a wide range of polymers which involve both natural and synthetic substances that are made up of poly (lactide-co-glycolide), poly(lactic acid), and many more. With respect to the delivery of drugs to various specific cells in the body, the composition of GNPs varies from one drug delivery system to other. These types of nanocarriers are also very cheap and economical when compared with those of other types of NPs. Gold NPs (GNPs) are said to be the most extensively studied NPs and applied most importantly in Cancer Biology and Medicine. Gold NPs have varied biomedical applications, such as genomics, immunoassays, microorganism detection and control, photothermolysis of cancer cells, targeted drug delivery, optical imaging,

and monitoring of biological cells and tissues by resonance scattering. Au nanospheres were the first GNPs to be discovered, which were followed by various forms, such as nanorods, nanoshells, and nanocages (Schrag et al. 2006).

Protein NPs are considered to be most efficient colloidal drug carrier systems which primarily affect the drug targeting system with the help of modified protein NPs by reducing drug toxicity. Prevention of enzymatic degradation by these NPs was considered to be a vital merit for these NPs. These types of NPs are already being extensively used as pharmaceutical carriers in various cancer therapies. Parenteral, peroral, and ocular types of administration are conducted using protein NPs in order to deliver large and small biological molecules.

Lipid NP systems are also extensively being used in cancer therapy in the form of solid NPs (SLNPs) and nanostructured lipid carriers (NLCs). SLNPs were formed from a single purified lipid which forms a crystalline lattice structure which was helpful for the incorporation of small molecular drugs. They are said to have a unique size-dependent properties which made them to have varied biomedical applications. Quantum dots are also known as fluorescent semiconducting nanocrystals, which have several biomedical applications, such as biolabels, sensors, light emitting diodes, and medicine (Schrag et al. 2006).

The most frequently used nanocarriers in recent times have varied types of nanogels as they have gained much importance in recent times due to their excellent medical applications and properties such as biocompatibility, which is vital for clinical treatment of many types of cancers. Due to their high porosity, nanogels are widely utilized as reaction vectors in preparation of hybrid NPs, which are helpful in capturing metal NPs like Fe_3O_4NPs (Schrag et al. 2006).

Nanomaterials can be classified into four types, that is, zero dimensional (e.g., NPs, QD), one dimensional (e.g., nanotubes, nanowires), two dimensional (e.g., ultrathin films), and three dimensional (e.g., nanocrystal grains and clusters). There are various applications of nanomaterials which are illustrated in Fig. 3.1, such as biosensors, wound healing, and target imaging (Langer 2001).

3.1.5 Disease Therapy

The main role of utilizing autoimmune disease therapy is to block pathological infection without disturbing the immunity of our body toward varied infections. Various studies have been performed, which gave a clarification about the role of nanoparticles in autoimmune disease therapy (Agarwal 2006). Shyam et al. (2013) briefed the importance of carbon nanotubes and graphenes in biomedical applications which minimized drug loss and drug degradation. This study also proved the uniqueness of NPs as they were functionalized with specific biomolecules. Serra and Santamaria (2018) stated that NPs act as vehicles for immune modulators and also for antigen delivery to APCs. This study also revealed that NPs act as direct T-cell targeting compounds. Several studies have shown the importance of certain

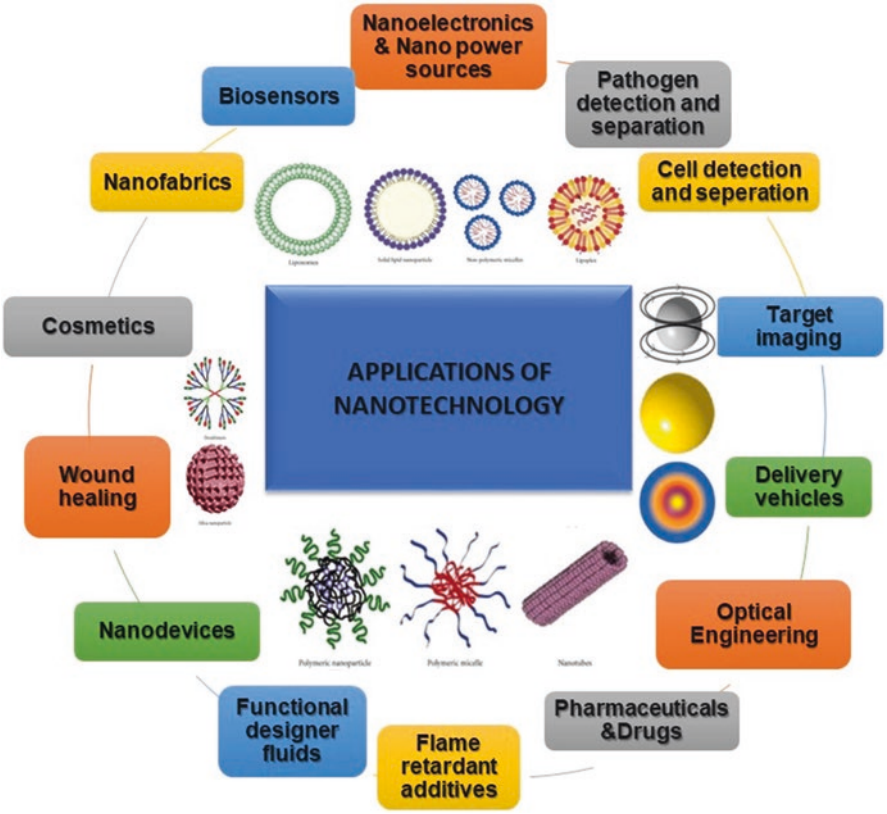

Fig. 3.1 Applications of nanotechnology

types of nanoparticles as effective carriers of therapeutic drugs to the target cells of the body.

3.2 Recent Developments in the Treatment of ND Using Nanomaterials

Neurodegenerative disorders are mainly caused due to poor delivery of therapeutic drugs into the brain. Blood–brain barrier is considered to be a functional barrier which permits only selective essential nutrients to the brain, which made us to know the importance of designing an effective route of administration of drugs to treat neurodegenerative diseases. Akinc and Battaglia (2013) briefed about the importance of intranasal drug delivery system of lipid NPs to treat neurodegenerative disorders, such as Parkinson's disease and Alzheimer's disease, as it was said to be a noninvasive type of drug delivery system and directly provides drug to the brain

through intra- and extraneuronal pathways. This study has also proved that most of the therapeutic drugs have failed to cure neurodegenerative diseases. Intranasal drug delivery with the help of lipid NPs was said to be the most effective and simplest methodology of drug administration as utilization of lipid NPs helps by enhancing bioadhesion to nasal mucosa and providing protection to the encapsulated drug.

Amanzadeh et al. stated that drug delivery into brain to treat temporal epilepsy can be increased by preparing PEGylated PLGA nanoparticles of epigallocatechin 3-gallate with the help of double emulsion method, and along with this immune histochemistry, cytotoxicity and behavioral tests were carried out in order to know the efficiency of drug delivery of these types of nanoparticles (NPs). The results of these studies depicted that monodisperse NPs with average size of about 169 nm and encapsulation efficiency of 95% were nontoxic. Neurotoxicity studies were also carried out, which showed decrease in neuronal death and neuroinflammation. Thus, this study proved that epigallocatechin 3-gallate PEGylated PLGA NPs were found to be the best alternative for therapeutic drugs, such as phenytoin and carbamazepine, which are being used to treat temporal epilepsy.

Another study by Sonvico et al. (2018) briefed about intranasal delivery of insulin using NPs would help in the treatment of Alzheimer's disease as it showed promising results in clinical trials. The biocompatibility and stability of these NPs proved to be helpful for the design of an effective intranasal drug delivery system.

3.3 Cell-Mediated Delivery of Nanocarriers to the Brain

A distinct case of the vehicle-mediated CNS drug delivery employs specific cell carriers that can incorporate micro- and nanocontainers loaded with drugs and act as perfect Trojan horses by migrating across the BBB and carrying drugs to the site of action. It is documented that AD, PD, stroke, ALS, and HAD have in common an inflammatory component. The process of inflammation is characterized by extensive leukocytes (neutrophils and monocytes) recruitment. Our knowledge of the function of MP has evolved considerably since Ilya Mechnikov, over a century ago, discovered an inflammatory cell type in starfish larvae capable of engulfing foreign objects (Kim 2007). The past century has realized that a great deal of progress in understanding the role MP plays in immunity and most notably in the CNS. Macrophages orchestrate intracellular killing of pathogenic microbes, antigen presentation, and secretion of biologically active factors, as well as mediation of pathological processes (Teleanu et al. 2019). Underlying such cellular functions is inflammation; the same response often proves detrimental in localized and systemic diseases, including those operative in neurodegenerative disorders. Inflammatory processes are engaged in attempts to eliminate the invader along with clearance of damaged tissue.

With regard to the nervous system neuroinflammation, perpetrated through activation of brain MP along with other glial elements, including astrocytes and endothelial cells, may act through paracrine pathways to accelerate neuronal injury in

highly divergent diseases, such as AD, PD, stroke, ALS, HAD, and spongiform encephalopathies or prion-mediated neurodegeneration. The biological processes causing inflammatory responses into a neurotoxic state, thus, are common links to many other neurodegenerative diseases (Robichaud 2006). As the role of the immune system is clarified, new opportunities for amelioration of the neurodegeneration from neurotoxins produced by the immune system will become critical for early treatment. In neurodegenerative diseases, CNS inflammatory infiltrates are complex and multifaceted. The initial responders or the MP cell elements of innate immunity set up a cascade, which later involve the activation and recruitment of the adaptive immune system and ultimately tissue destruction. MP and T cells possess the unique property of migrating toward the site of inflammation through the processes known as diapedesis and chemataxis. Their combat arsenal consists of uptake of the foreign particle, producing toxic compounds, and liberation of substances stored in intracellular vesicles via exocytosis. Therefore, these cells can be used for cell-mediated CNS drug delivery when loaded with a drug and administered into the blood stream (Tyler and Federoff 2006).

There have been many findings by various researchers all over the world about the importance of NPs in varied biomedical applications. When it comes to treatment for neurodegenerative diseases, there is an alarming need to know the combinational effects of neurotoxicology and nanotoxicology. Thus, Bencsik et al. (2005) stated the importance of evaluating both neuro- and nanotoxicity, as NPs may affect the human brain health due to large amount of industrial production. This study also briefs about the essential need for innovating specialized tools to carry out an effective evaluation of both neuro- and nanotoxicology.

There are certain types of cytokines that help in preventing neurodegenerative diseases from attacking our body. The study by Davis et al. (2005) helped in evaluating biological activity of NPs which contained leukemia inhibitory factor (LIF). Initially, packaging of LIF was done in nanoparticles which comprise poly (ethylene glycol)-poly(lactic acid) (PEG-PLA), which lead to the formation of LIF-loaded NPs which were also known as NanoLIF. In order to increase the cytokine delivery to inflammatory macrophages, the surface of NanoLIF was made to undergo changes with the help of CD11b antibody, which targets the activated peripheral macrophages. ELISA was used to evaluate the release of cytokine from NanoLIF. M1 murine leukemia cell proliferation assay was helpful in measuring the biological activity of NanoLIF. The results from this study showed that the average diameter of NanoLIF was found to be 30 nm and had a neutral surface charge. This study also proved that NanoLIF could release LIF at rapid rate at about 0–6 h after incubation at 370°C initially and slows down within 72 h. This study was very much helpful in proving that NanoLIF and CD11b-NanoLIF were responsible for minimizing M1 cell proliferation in a larger amount and thereby blocking neurodegenerative diseases from attacking our body.

In one of the studies, various in silico techniques were employed to make the therapeutic drugs which are commonly employed in the treatment of neurodegenerative disorders to cross the blood–brain barrier (BBB) using nanotechnology (Smith and Gumbleton 2006). There is a vital need in designing a methodology that

would exploit the BBB cells at molecular level without disturbing the normal functioning of the barrier. Receptor and adsorptive-mediated transcytosis were considered to be one of the most valuable mechanisms which would help in transport of nanomaterials from the blood to the brain by passing through BBB. Electrostatic interaction of a ligand including the charges expressing at the luminal surface of endothelial cells is said to be completely dependent on adsorptive-mediated transcytosis (Smith and Gumbleton 2006).

There have been many studies in which combination of various drugs with nanomaterials has helped in treating various neurodegenerative disorders. The study by Gobbi et al. (2005) proved that nanoliposomes (NL) when functionalized with phosphatidic or with cardiolipin was found to be helpful in the treatment of Alzheimer's disease (AD). Along with the use in treatment for AD, NPs can also be used in protecting neuronal cells against oxidative stress. Due to toxic effects, metal chelators are not being employed in recent times to prevent oxidation damage, and as NPs are free from toxicity, these can be used as a best alternative to metal chelators.

Nanotechnology has also produced in vitro diagnostic tools for varied neurodegenerative diseases like AD by measuring known pathogenic markers, such as tau proteins and ADDLs of human cerebral spinal fluid (CSF). In one of the studies, Georganopoulou et al. (2005) used bio-barcode of GNPs in determination of ADDL concentration in order to diagnose the disease early. A study introduced another in vitro technique in which quantum dots were conjugated with streptavidin which lead to easy recognition of APP, which was found to be highly sensible when compared with that fluoroimmunoassay (Flachenecker 2006).

Parkinson's disease [PD] is said to be a progressive neurological disorder generally affecting old age people. Nanotechnology plays a major role in release of dopamine from brain, which is helpful in treating PD. Trapani et al. (2005) prepared chitosan NPs along with which DA was adsorbed on to the external surface. Then, they were administered intraperitoneally into the mice whose results showed less cytotoxicity when they were compared with that of only DA-administered mice. There are in vivo diagnostic techniques for early detection and diagnosis of PD with the help of nanotechnology. Neumann (2006) designed a highly sensitive immunosensor for early PD detection with the help of Au doped–TiO_2 nanotubes arrays.

CNS injuries are commonly caused during neurodegenerative disorders, which are generally followed by accumulation of reactive oxygen species. Thus, in order to minimize these levels, fullerenes were utilized, which would act as radical sponges which have the capacity to incorporate multiple radicals in a single molecule, which thereby leads to removal of superoxide oxygen radicals by dismutation catalytic mechanism. One of the main reasons excluding BBB for the poor treatment for neurodegenerative disorders is that the therapeutic drugs which are being used for treatment are only symptomatic as they do not reduce the progressive pathological condition of the patient (Pardridge 2005). Tosi et al. (2008) in a review study stated that nanomedicine for every CNS disorder does not exist, which clearly shows that, however, nanomedicine is useful for treatment and early diagnosis of neurodegenerative disorders. Thus, there is an alarming need to try to produce new

nanomedicines that could be metallic, polymer, or biosynthesized, which would be helpful in treating these kinds of heinous disorders.

The transport mechanisms that are involved in BBB could be manipulated which were proved by various studies, which were related to kinetic flux that disclosed unidirectional, concentration dependent movements of compounds across the BBB. Various studies have reported that polymeric NPs (PNPs) have the capability of delivering varied CNS drugs, such as Doxrubicin, which proves that PNPs could play a vital role in the treatment of different CNS disorders in near future (Gaillard et al. 2005).

In a recent study by Huang et al. (2006), lactoferrin was initially made to modify NPs which were then injected into rats suffering with PD, which resulted in showing increased locomotor activity and enhanced DA levels in rats suffering with PD. Vinogradov et al. (2012) tried to create a new combination with NPs by encapsulating oligonucleotides in nanogels, which led to absorption of oligonucleotides into brain through BBB by reducing the amount of absorption of oligonucleotides in liver and spleen.

Nanosuspensions were considered to be excellent nanocarriers because of their effective properties, which were evaluated in various studies done till date on simplicity, high drug loading capacity, and application to various number of CNS drugs. Electrical stimulation is another type of methodology which is being adopted in recent times to treat CNS disorders (Lehericy 2007). In a recent study, carbon nanotubes were used to enhance chronic electrical stimulation of CNS, which would be helpful in treating neurodegenerative disorders (Kingsley 2006).

There have been various studies which were adopted to modify the concept of electrical stimulation in CNS with an aim to prevent these diseases, and by doing these studies, they could identify that nanofibers would also be helpful in treating neurodegenerative diseases. Silva (2006) reported that carbon nanofiber-based electrode arrays had the capability to provide both physical substrate and molecular signals when they were injected at the degenerative sites of brain.

Polymeric nanomicelles have also been one of the promising type of nanomaterials which can be utilized for treatment in CNS disorders in near future as some of the studies have proved that nanomicelles can transport DNA to the CNS by performing both in vitro and in vivo studies, but there is still a lot of scope to exploit this type of nanomaterial which has the capability to produce several biomedical applications (Wang et al. 2008).

A study has reported that N-butylcyanoacrylate (PBCA) NP combined with clioquinol (CQ), which is said to be a quinoline derivative, was injected into a transgenic mice, and the results showed that this type of NPs which were used to deliver clioquinol to the brain helped in solubilization of beta-amyloid plaques which were responsible for the cause of AD, and thus, it led to inhibition of these structures, which further caused prevention of AD. Similarly, there have been many studies which are helpful in proving that PBCA NPs help in transporting varied number of drugs to the CNS in an effective manner (Banks 2002).

There have been several studies which reported that accumulation of more number of metal ions which actually gets increased with age was said to be responsible

for causing AD or enhancing their effects in patients suffering with AD. Thus, in order to prevent this, Cui et al. (2005) reported that when Cu (I) chelator d-penicillamine was conjugated with NPs, it leads to reversing of metal-induced precipitation of B-amyloid protein, which finally lead to the prevention of AD.

The study by Kogan et al. (2006) reported that GNPs can also be useful in destruction of B-amyloid plaques by incorporating them into B-amyloid fibrils and then exposing them to weak microwave fields, which resulted in producing energy which was six times lower than that of energy produced from cell phones and proved that it was safer to use in the presence of healthy cells.

Ritchie et al. (2015) reported that Thioflavin-T NPs (ThT) was helpful in detection of AD by identifying B-amyloid in senile plaques of AD. The above study was followed by another study by Härtig et al. (2017) who tried to induce ThT NPs comprising of PBCA into the brains of transgenic mice, and the results showed that photoconversion of ThT occurred from the fixed tissues, and transmission electron microscopy proved the presence of nanocapsules in microglia and neurons of mice. The delivery of ThT from nanocapsules was observed with the help of confocal microscopy.

Several studies have shown that varied number of peptides and proteins which are emerging as nanobiomaterials have the capability of self-assembling themselves into various nanostructures such as nanotubes, nanovesicles, helical ribbons, and three-dimensional scaffolds (Tamai and Tsuji 2000). Stupp et al. (2014) injected a nanobiomaterial into a lab mice which was suffering with a spinal cord injury, and the results showed that the mice which was initially made to get paralyzed was able to walk after injecting with the nanomaterial by regenerating the damaged neurons. This was only possible because of self-assembling property of these nanobiomaterials.

In any nanomaterial, self-assembling property plays a key role in preventing AD-like disorders. Thus, there have been several studies made on GNA, which is a derivative of DNA that possessed the self-assembling property along with some additional properties which were lacking in DNA, such as antiamyloid activity and formation of mirror image structures, which were helpful in synthesis of various therapeutic proteins that are useful in vivo gene delivery of AD treatments (Saltzman 1999).

3.4 Nanogels

Nanogels are considered as nanosized networks of cross-linked polymers that often combine both ionic and nonionic chains, such as polyethyleneimine (PEI) and PEG or poly (acrylic acid) and Pluronic®. Such networks swell in water and can be incorporated through ionic interactions of oppositely charged molecules, such as oligonucleotides, siRNA, DNA, proteins, and low molecular mass drugs (Broadwell and Sofroniew 1993).

Transport of oligonucleotides incorporated in nanogel particles across an in vitro model of the BBB was recently reported (Weissig 2006). Notably, nanogels decreased the amount of degradation at oligonucleotides during their transport in BMVEC. To further enhance delivery across the BBB, the surface of nanogels was further modified by either transferrin or insulin.

In vivo studies suggested that nanogel increased brain uptake of oligonucleotides while decreasing its uptake in liver and spleen. Overall, nanogels are promising carriers for CNS drug delivery, although they are in relatively early stages of development (Bocti 2006).

3.5 Other Nanomaterials

Carbon nanotubes attracted attention in nanomedicine although there were serious concerns regarding their safety. Continuous electrospun nanofibers are also unique as they depict nanostructures in two dimensions and macroscopic structures in another dimension. They are safer to be manufactured than carbon nanotubes and possess less risk of air pollution. Electrospun nanofibers of a degradable polymer, PLGA, loaded with dexamethasone have been used for neural prosthetic applications (Dhib-Jalbut 2006).

A conducting polymer, poly(3,4-ethylenedioxythiophene), was deposited to the nanofiber surface, and the coated nanofibers were then mounted on the microfabricated neural microelectrodes, which were implanted into brain. The drug was released by electrical stimulation that induced a local dilation of the coat and increased permeability (Khachaturian 2006). Simpkins and Bodor (2014) proved that redox-based drug delivery nanosystems were successful in delivering and targeting release of DA into the brain through BBB, which generally blocks DA from entering the parenchymal layer of brain, and thus, these types of nanosystems are helpful in treatment for patients suffering with PD.

Gene therapy with help of nonviral vectors has proved to be effective in the treatment of PD, but various studies in recent times have also proved that NP-based gene therapy can also be developed for treating PD patients in a more safer and economical way. Wang et al. (2006) reported that NP-based gene therapy demonstrated lesser side effects than other types of gene therapy. This study also proved that this type of gene therapy resulted in showing enhanced clinical symptoms and abnormal metabolism from baseline when measured with the help of tomography. Ramanathan et al. (2018) conducted an experiment on animal model by condensing DNA plasmids into NPs which would help in repairing defective genes and thus finally prevent neurodegeneration in those animals by showing improved symptoms in them which proved that NP-based gene therapy effectively cures neurodegenerative disorders like PD.

3.6 Nanorobots

Nanorobots are biomachines which can algorithmically respond to stimulation and also have the capability of actuation, sensing, signaling information, processing, and intelligence. In a recent study, Prinster (2006) reported that stem cell therapy is useful in preventing the attack of PD by conducting experiments on rats into which they implanted stem cells directly into brain which lead to regeneration of striatal neurons and partial recovery of motor deficit which are actually related to deficiency of PD.

3.7 Conclusion

Nanotechnology is very much useful as it possesses many biomedical applications in drug delivery, separation technology, nanoelectronics, catalysis, and most importantly for treatment of neurodegenerative disorders. Polymer and protein NPs are said to be very much useful as an excellent nanocarriers to treat various CNS disorders. Several studies have proved that quantum dots had varied biomedical applications as biolabels, sensors, light-emitting diodes, and medicine. One study has proven that NP-based gene therapy can also be developed for treating PD patients in a safer and economical way. In another study, it was proved that lipid NPs when given by intranasal drug delivery system were helpful in treatment for various neurodegenerative disorders such as AD and PD. Some of the studies also proved that NPs act as vehicles for immunomodulators and also for antigen delivery to APCs. One of the studies stated that silver nanoparticles can be produced by following green synthesis in which reduction of silver nitrate occurs in the presence of water-soluble polymer such as poly-L-lysine. Thus, by this review, we can clearly understand the importance of nanotechnology in preventing NDs from attacking our body, and there is still a lot of scope to analyze and find more new biosynthesized NPs which have the capability to cure neurodegenerative disorders in near future.

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Chapter 4
Nanobiotechnology in Neurodegenerative Diseases

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Abstract "Neurodegenerative disease" is a term for a variety of disorders that primarily affect neurons in the human brain and spinal cord. These diseases are presently incurable and result in progressive degeneration or death of nerve cells, resulting in impaired movement (ataxia, Parkinsonism, paresis) or mental functions (dementia). The most common neurodegenerative diseases include Parkinson's disease and other parkinsonian syndromes, Alzheimer's disease and other non-Alzheimer's dementias, Friedreich's disease and other spinocerebellar atrophy, amyotrophic lateral sclerosis, and other diseases manifesting symptoms such as restriction of free movement, tremor, chorea, dystonia, myoclonus, other abnormal movements, dementia, and other cognitive disorders. Neurodegenerative diseases are highly prevalent and are among the most serious diseases in terms of health and socioeconomic impact. These diseases are not limited to older age groups, but affect also children and adults of working age. The current therapies cannot cure the diseases; they only ameliorate or relieve symptoms. All employed drugs have their targeted site of action in the central nervous system; thus, overcoming the blood–brain barrier is a necessity. Nanotechnology provides a new dimension and new properties to all materials and, in particular, allows central nervous system targeting of nanoscale formulations with increased brain permeation, and it is, thus, widely used for the production of a new generation of pharmaceuticals and theranostics with improved drug bioavailability, reduced undesirable side effects, minimized

This chapter is sincerely dedicated to the memory of Professor Karol Matulay (1906–1998), nestor of Slovak psychiatry and neurology.

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nonspecific uptake, and specific targeting to certain target cells. This chapter presents a comprehensive overview of recent findings in the field of investigation and application of nanoformulations tested/used for the alleviation or treatment of Parkinson's disease, Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis, Huntington's disease, and Wilson's disease as well as nanosensors applied for diagnostics or a treatment monitoring of neurodegenerative diseases.

Keywords Nanoparticles · Nanoformulations · Central nervous system · Neurodegeneration · Targeted delivery · Pharmaceuticals · Nanosensors · Theranostics

4.1 Introduction

Worldwide, approximately 1.5 billion people are suffering from various central nervous system (CNS) disorders, including neurodegenerative diseases such as Parkinson's disease (PD), Alzheimer's disease (AD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), stroke, HIV dementia, and others. Efficacious treatments for most of these are lacking, often due to the inability of drugs to pass effectively into the CNS; therefore, the development of effective targeting strategies to deliver drugs into the brain is of utmost urgency (Barnabas 2019). The number of people suffering from these disorders is increasing, with projections speaking of 131.5 million patients expected to be affected by 2050 just by Alzheimer's disease, the most common dementing disorder of the elderly (Alzheimer's Disease International 2015). As many of these disorders are age associated (Masters et al. 2015; Coyle-Gilchrist et al. 2016), this rise in prevalence is (at least in part) driven by the aging of the global population, though numerous other factors likely contribute to incidence, such as a sedentary lifestyle, diabetes, hypertension, cranial trauma, or the increasing amount of pollutants (e.g., pesticides or toxic metals) in the environment (McKee et al. 2013; Baumgart et al. 2015; Chin-Chan et al. 2015). While a majority of neurodegenerative disorders arise sporadically, a fraction is caused by mutations that commonly display early onset and excessively abundant pathology (Blennow et al. 2006; Sieben et al. 2012).

As CNS disorders inevitably affect the individual's ability to function independently, their devastating impact is not limited to the patient alone, but affects their caregivers and society as a whole, incurring massive healthcare costs (Wimo et al. 2011), and leading to a deterioration of health in caregivers as well (Clark et al. 2007). This makes their treatment an important unmet need.

Presently, the treatment of neurodegenerative disorders is mostly symptomatic, such as correction of neurotransmitter imbalances in AD (Lyketsos et al. 2006; Atri et al. 2013) or PD (Schulz et al. 2016). Treatments that would affect the underlying

pathology and halt the progression of neurodegenerative disorders are generally absent.

Nanoparticles (NPs) and various nanosystems are widely used in various sectors of human activity (Jampílek and Kráľová 2015, 2017a, b, c, 2018a, b, c, 2019a, b, c, d, e; Pentak et al. 2016; Pisárčik et al. 2016, 2017, 2018; Vaculíková et al. 2016a, b, 2019; Jampílek et al. 2019; Kozik et al. 2019). Nanosystems are widely used as drug carriers for controlled release and/or targeted delivery of drugs, therapeutic proteins, genes, etc. NPs as drug carriers are extensively studied as a direct drug delivery tool to the central nervous system (CNS) through the blood–brain barrier (BBB) (Ljubimova et al. 2017; Sun et al. 2017). The uptake of nanodelivery systems into the brain was hypothesized to occur via adsorptive transcytosis and receptor-mediated endocytosis (Yang et al. 2010a; Wong et al. 2012; Johnsen et al. 2017), with particle size, surface affinity, and stability in circulation being important factors influencing the brain distribution of these nanoformulations (Masserini 2013; Simko and Mattson 2014; Nair et al. 2018; Vargas-Osorio et al. 2019). Rapid development of nanomedicine has offered new opportunities in treatment strategies. Kang et al. (2018) classified novel disease hallmarks incorporated in emerging nanoplatforms, described effective delivering strategies to improve the treatment of CNS disorders while decreasing undesirable side effects, and focused also on their implications for clinical practice. The role of nanoformulations (e.g., a PEGylated immunoliposome encapsulated tyrosine hydroxylase expression plasmid effectively crossing the BBB and restoring motor behavior in rat model, a $C_{60}(OH)_{24}$ fullerene derivative showing superb free radical scavenging activity and antioxidant potential, or nanoscale CeO_2 particles acting as reactive oxygen species (ROS) scavenger and thus decreasing oxidative stress) in the remediation of PD was reviewed by Mandal et al. (2018) and Siddiqi et al. (2018). Kuo and Rajesh (2018) summarized the recent findings related to drug delivery systems suitable to improve the efficacy of drug/gene in PD treatment. An overview of nanocarriers used to improve the treatment of neurodegenerative disorders, such as PD and AD, through nose to brain targeting that can bypass the BBB and target drugs directly to the brain was presented by Md et al. (2018).

This comprehensive chapter is focused on the effects of nanoscale formulations, covering a wide range of therapeutics applied in the treatment or palliation of Parkinson's disease, Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis, Huntington's disease, and Wilson's disease. Nanosensors applied for diagnostics or a treatment monitoring of neurodegenerative diseases are mentioned briefly as well.

4.2 Pathology, Phenotype, and Biochemistry

The brain has a limited range of responses to injury, generally manifesting as loss of function in affected regions that is proportional to the severity of the pathological changes. Thus, the most often amnestic phenotype of AD and its nonamnestic varia-

tions come to be, and thus arise the specific phenotypes of frontotemporal dementias, PD, atypical Parkinsonism syndromes, and other neurodegenerative disorders. Taking AD as an example, as pathology mounts, the clinical state progresses from asymptomatic to discreet deficits, to mild cognitive impairment, and to full-blown dementia. Multidomain cognitive impairment beyond amnesia manifests once there is sufficiently severe pathology outside the medial temporal lobe. Analogously, lesions in the frontal and anterior temporal lobes are responsible for the dysexecutive and behavioral changes in frontotemporal dementia (FTD) (Warren et al. 2013).

Only once one examines the pathology underlying these disorders does the true diversity of neurodegeneration come to light. Almost universally, aggregates of pathologically modified proteins are found, whether extracellularly, or inside neurons as in the case of AD (Schulz et al. 2016), or inside astrocytes in corticobasal degeneration (CBD) or progressive supranuclear palsy (PSP) (Josephs et al. 2011), or in oligodendrocytes in argyrophilic grain disease (Grinberg and Heinsen 2009).

As a combined proteinopathy, AD is characterized by the concomitant spreading of intracellular aggregates of protein tau and extracellular amyloid-β (Aβ) deposits (Braak and Braak 1991) (Fig. 4.1). A broad range of non-AD tauopathies similarly displays pathology composed of the microtubule-associated protein (Novák et al. 2018a). The morphology and distribution of lesions vary, manifesting as executive dysfunction and behavioral abnormalities in chronic traumatic encephalopathy (McKee et al. 2013) or the behavioral variant of FTD (Warren et al. 2013): gaze palsy, atypical Parkinsonism and varied cognitive impairment in PSP (Respondek and Hoglinger 2016), language deficit in the nonfluent variant of primary progressive aphasia (Josephs et al. 2011), or a range of deficits including alien limb syndrome, asymmetrical Parkinsonism, behavioral changes, and cognitive impairment in CBD (Armstrong et al. 2013), to name a few.

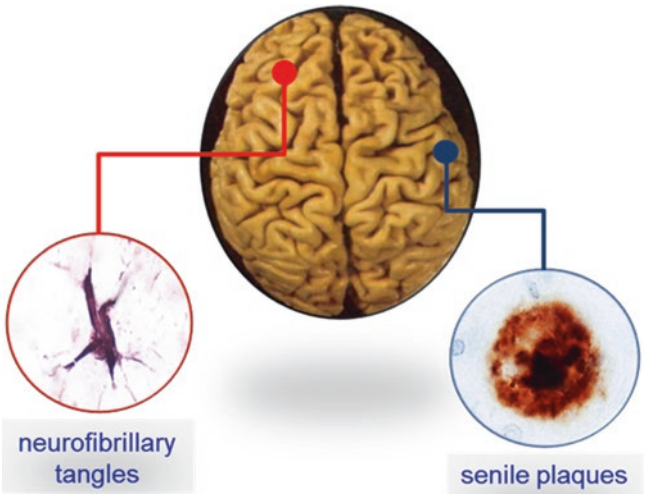

Fig. 4.1 Brain of patient with Alzheimer's disease

Lesions composed of the transactive response DNA-binding protein (TDP-43) often manifest as behavioral variant frontotemporal dementia as well, making differential diagnosis between frontotemporal lobar degeneration (FTLD)-tau and FTLD-TDP challenging. TDP-43 pathology is commonly associated with the phenotype of semantic dementia, an asymmetric neurodegenerative disorder affecting predominantly the anterior temporal pole, and leading to the loss of association between words and their meaning; also, FTD with an accompanying motor neuron disorder most often displays underlying TDP-43 lesions (Gorno-Tempini et al. 2011; Sieben et al. 2012). The picture of ALS is dominated by the motor neuron disorder and accompanying TDP-43 (or, in rarer cases, superoxide dismutase (SOD)-1) lesions in motor neurons; the fact that the disorder is commonly accompanied by cognitive and behavioral changes highlights that ALS and frontotemporal dementia with motor neuron disease (FTD-MND) are parts of a spectrum (Hardiman et al. 2017). Hippocampal sclerosis with TDP43 lesions constitutes a common finding especially in older patients and constitutes a confounding factor in the radiological assessment of hippocampal atrophy in the diagnosis of AD (Nelson et al. 2011). Recent research shows that limbic-predominant age-related TDP-43 encephalopathy (LATE) constitutes another important differential diagnosis of AD especially in the oldest-old; the disorder is characterized by TDP43 lesions in neurons, oligodendroglia, and astrocytes in the amygdala, hippocampus, and middle frontal gyrus; its phenotype mimics AD to a certain degree (Nelson et al. 2019).

A smaller subset of FTD cases have inclusions of fused-in-sarcoma protein (FUS) as the underlying pathology and can present with or without MND (Mackenzie et al. 2010).

Meanwhile, in PD, PD with dementia (PDD), and dementia with Lewy bodies (DLB), the salient lesions are composed of α-synuclein; their distribution influences whether clinically, the disorder presents predominantly with motor symptoms, dementia with hallucinations, or a blend thereof (Braak and Del Tredici 2017). Lesions of α-synuclein in oligodendrocytes are also at the root of multiple system atrophy (MSA), a disorder presenting with progressive autonomic and motor dysfunction (Stefanova and Wenning 2016).

Accumulation of polyglutamine strands of variable length due to expanded CAG repeats on chromosome 4 led to the classic picture of Huntington's disease (Walker 2007).

No mention of neurodegenerative disorders would be complete without the mention of prion disorders – Kuru, fatal familial insomnia, Gerstmann–Sträussler–Scheinker disease, Creutzfeldt–Jakob disease (CJD), or its bovine-derived variant vCJD (Prusiner 1998). Unlike most of the diseases described above, these disorders have abundant counterparts in the animal kingdom – scrapie in sheep, chronic wasting disease in deer, and the widely publicized bovine spongiform encephalopathy (BSE) in cattle (Collinge 2016). Perhaps most importantly, these disorders introduced the concept of template-mediated conformational change to the field of neurodegeneration research (Novák 1994; Clavaguera et al. 2009). This mechanism is found to be the unifying feature of pathology propagation in prionoses, synucleinopathies, and tauopathies (Brundin et al. 2010; Novák et al. 2011); its variations

are responsible for differences in the speed of progression (Aoyagi et al. 2019) and in the lesion distribution—and thus the resulting phenotype of the disorder.

The picture of neurodegeneration is complicated by the fact that, especially in older patients, different pathologies coexist. Rare is the nonagenarian without a modicum of both neurofibrillary and TDP43 pathology in their brain (Spires-Jones et al. 2017). Vascular pathology commonly coexists with AD neuropathological changes, as do α-synuclein lesions (Jellinger and Attems 2015). Generally, "pure" cases are found in younger patients, where one given neuropathological process is aggressive enough to overcome the brain's defenses early, before the onset of other age-associated pathologies (Attems and Jellinger 2014). The implications of this process are two-fold. First, the clinical phenotype observed in a given patient is often the product of several types of lesions, as is the progression rate. Second, this complicates development of disease-modifying therapies, as, for example, a therapy aimed specifically at neurofibrillary tau pathology is none too likely to affect TDP43 lesions. In a patient with a complex neuropathological picture, even if such a therapy halts the progression of neurofibrillary degeneration completely in its tracks, the patient may continue to decline, albeit at a slower pace, as the other types of lesions accrue. Though the example may be farfetched, this calls for either personalized interventions based on the patients' neuropathological processes or broad-spectrum agents that affect multiple types of neurodegeneration at once. In the context of drug development and clinical trials, thorough diagnostic workup of recruited patients, including possibly several biomarker assessments, becomes a necessity as to ensure enrolment of patients who possess fairly pure forms of the disorder targeted by the investigational medicinal product.

Finally, the primary neuropathology is often accompanied by other effects, such as neuroinflammation and dysfunction of the brain's immune system (Streit et al. 2009; Streit and Xue 2014; Heneka et al. 2015), and impaired blood–brain barrier function (Majerova et al. 2018).

A fundamental theme in neurodegenerative disorders is the accumulation of a pathological version of an endogenous protein, which is accompanied by loss of function and toxic gain of function. The protein is, furthermore, heavily modified in comparison to its healthy counterpart. This is, perhaps, best illustrated on tau protein pathology. In disease, the protein acquires a multitude of modifications – truncation (Novák et al. 1989), hyperphosphorylation (Grundke-Iqbal et al. 1986), glycosylation (Wang et al. 1996), glycation (Ledesma et al. 1994), ubiquitination (Mori et al. 1987), nitration (Horiguchi et al. 2003), or polyamination (Tucholski et al. 1999). Of these, phosphorylation and truncation appear most important, as they lead to loss of important functional domains, global conformational change of the protein, and acquisition of proaggregant properties (Wischik et al. 1988; Novák et al. 1991; Novák et al. 1993; Berry et al. 2003; Gamblin et al. 2003; Rissman et al. 2004; Binder et al. 2005; Sevcik et al. 2007; Kovacech and Novák 2010; Kovacech et al. 2010). The exact combination of posttranslational modifications then influences the final shape of the aggregates, and thus their behavior, with differences of posttranslational modification patterns resulting in as vastly different lesion distri-

bution patterns and phenotypes as AD and PD (Fitzpatrick et al. 2017; Falcon et al. 2018).

Infectious oligomers and aggregates are formed as a result. The protein abandons its original purpose, and instead, it spreads from cell to cell via a range of mechanisms, generally not based on proximity, but rather connections between cells (Novák et al. 2011; Clavaguera and Hench 2015).

This mechanism proceeds with striking similarities in AD (Aoyagi et al. 2019), PD (Brundin et al. 2008), and TDP43 disorders (Ishii et al. 2017), and, obviously, prions (Prusiner 1998).

It is not farfetched to think that the posttranslational modifications and the resulting differences in the properties and shapes of infectious aggregates would explain the differential vulnerability of cell populations, very much like differently shaped keys open different locks. The strain concept long present in the prion field has been introduced into the field of dementia research (Frost et al. 2009; Brundin et al. 2010; Kaufman et al. 2016), facilitating the understanding of differences between disorders and variance within disorders as well.

The complexity of the abovementioned posttranslational modification patterns and strain properties pose unique challenges and offer specific opportunities. On one hand, the modifications set pathological proteins apart from their healthy counterparts and create by definition a novel epitope structure that allows selective targeting of these structures, for example, by immunotherapy, as to avoid off-target reactivity. The challenges are obvious – the possible combinations of modifications are truly countless, and certain pools of pathological molecules may lack certain epitopes, domains, or affinities, thus rendering them impervious to therapies aimed at these features. Common denominators of various strains can be identified – so was it recently shown that, despite the different tau strains that cause PD, CBD, PSP, and AD, certain epitopes are present in each of them, and throughout the pathological tau proteome (Novák et al. 2018b).

Efficacious treatment of neurodegenerative disorders will not only have to be based on an in-depth understanding of the above mechanisms, and properties of the various proteinopathies, but will have to overcome also challenges common to all CNS therapeutics – ferrying sufficient drug concentrations across the BBB (with the added challenge of it being damaged and/or dysfunctional), targeting them at the right cell populations, and engaging the pathology without detrimental off-target effects.

4.3 Agents for Relieving Parkinson's Disease

PD is the second most prevalent neurodegenerative disorder worldwide. It is primarily characterized by progressive deterioration of motor functions, with symptoms such as rigor, tremor, and bradykinesia, due to progressive degeneration of dopaminergic nigrostriatal neurons and dopamine loss. Further spreading of pathology leads stepwise to cognitive impairment, forgetfulness, and ultimately dementia.

Oxidative stress, inflammatory factors, and acetylcholinesterase (AChE) activity could be considered as crucial disease-inducing factors.

Amyloidogenesis of α-synuclein (α-Syn), a presynaptic neuronal protein that aggregates to form fibrils in neuronal cells of PD patients and results in the loss of dopaminergic neurons, is considered to be the cardinal pathological phenomenon related to this disease.

Beside accumulation of α-Syn protein aggregates, also oxidative stress and ROS-mediated mitochondrial dysfunction as well as neuronal cell death are the pathological hallmarks of PD (Akbar et al. 2016; Weng et al. 2018). As the majority of dopaminergic neurons present in the substantia nigra of PD patients is destroyed before the disorder is clinically diagnosed, PD diagnosis in early disease stages is essential for timely intervention and the opportunity to rescue dopaminergic neurons, which requires appropriate analytical methods (Aziz et al. 2019). An overview of the recent advances in micro- and nanomedical approaches for repairing dopaminergic neurons was presented by Torres-Ortega et al. (2019).

4.3.1 Antiparkinsonics Drugs

In general, antiparkinsonic drugs increase "dopamine" effect in CNS, that is, increase concentration of dopamine in CNS (enhancement of permeation through the BBB or inhibition of degradation enzymes), and act as receptor agonists or anticholinergic drugs.

4.3.1.1 Dopamine and DOPA-Decarboxylase Inhibitors

Positively charged small liposomes with dopamine (DA) hydrochloride increased its concentration in the brain and protected DA against degradation compared to levodopa (LD), as well as to a marketed formulation of LD and carbidopa, which is an inhibitor of peripheral aromatic L-amino-acid decarboxylase (Jain et al. 1998). Transferrin (Tf) functionalized DA-loaded liposomes with entrapment/encapsulation efficiency (EE) >35%, particle size approx. 180 nm, and zeta potential of +7.5 mV were found to be suitable for DA brain delivery and could improve benefits and reduce complications in PD patients undergoing long-term LD treatment (Lopalco et al. 2018). Noncoated-, CS (chitosan)-, or CS-glutathione (GSH) conjugate-coated liposomes with encapsulated neurotransmitter DA partially protected it from the autoxidation reaction in neutral/alkaline conditions, the best results being observed with CS-GSH conjugate-coated liposomes, which could be connected with DA localization in the core of the vesicles and its absence on the surface of these vesicles. It is worth mention that the mean diameters of CS-GSH conjugate-coated liposomes were pronouncedly lower than those of noncoated and CS-coated vesicles, and CS or CS-GSH-coated vesicles showed a slightly positive zeta-potential, while the surface charge of DA-loaded noncoated liposomes was

10.8 mV (Trapani et al. 2018). DA-loaded poly(lactic-co-glycolic acid) (PLGA) NPs cross the BBB in an in vivo model of PD and thus increase the level of DA and reduce dopamine-D_2 receptor supersensitivity. In addition, this nanoformulation did not demonstrate negative effects on heart rate, blood pressure, or brain and peripheral organ function (Pahuja et al. 2015).

As mentioned above, carbidopa is an inhibitor of dihydroxyphenylalanine (DOPA) decarboxylase and, thus, inhibits the peripheral metabolism of LD. Satheesh (2014) used a biopolymer isolated from *Sesamum indicum* for nanoformulation of carbidopa.

DA after administration is extensively metabolized, and it is not able to cross the BBB; thus, its precursor/prodrug levodopa is used instead of DA. LD crosses the BBB and is converted in the CNS into DA by DOPA decarboxylase. Ravani et al. (2015) prepared controlled release LD-loaded lipid-core nanocapsules (LNCs) that demonstrated slightly reduced maximal efficacy but a longer lasting action (up to 24 h) in 6-hydroxydopamine (6-OHDA) hemilesioned mice. It should be noted that slow-release formulations are very attractive to PD patients, as otherwise some of them have to take medicines multiple times during the day. Zhou et al. (2013) published that spherical LD-loaded PLGA NPs (256 nm particle size, EE 62.2%) showed enhanced delivery of LD to the CNS. PLGA-loaded LD methyl ester/benserazide-loaded microspheres releasing LD and benserazide in a sustained manner were more suitable for reduction of LD-induced dyskinesia in a rat model of PD (Yang et al. 2012a, b). Parthipan et al. (2019) described one-step fabrication of bicompartmental microparticles (MPs) as a dual drug delivery system for PD management, in which the first compartment contained a relatively hydrophilic amorphous polymer PLGA and the second one an entirely hydrophobic semicrystalline polylactide, and PD drugs LD and carbidopa were localized in these compartments with 4:1 ratio, similar to commercially available tablets. These compartmentalized MPs simultaneously released >80% of both PD drugs within 5 h, suggesting that they could be potentially applied as dual drug delivery system. CS-coated LD nanoliposomes significantly decreased abnormal involuntary movement in rats in comparison to LD treatment (Cao et al. 2016). Intranasal administration a thermoreversible pluronic PF 127 gel with the CS NPs loaded with LD led to a higher concentration of LD in the CNS than drug dispersed in plain pluronic gel (Sharma et al. 2014). Tween-80-coated nanocomposites of LD incorporated in Zn/Al-layered double hydroxides (Zn/Al-LDH) showed slower drug release and improved viability of PC12 cells in comparison to nanocomposite without coating (Kura et al. 2013, 2014a, b). Carboxylated single-walled carbon nanotubes (SWCNTs) with LD demonstrated slow release of the drug with a release period over more than 20 h. In addition, this nanoformulation did not affect the cell viability of PC12 cells (Tan et al. 2015). Ngwuluka et al. (2015) described polymeric nano-enabled gastroretentive LD-loaded drug delivery systems with zero-order drug release suitable for a PD delivery system. Chlorotoxin-modified stealth liposomes (ClTx-LS) encapsulating LD significantly enhanced the uptake of liposomes by brain microvascular endothelial cells in vitro (Xiang et al. 2012). Using an 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD mouse model, it was found that

administration of LD-loaded crystalsomes led to a greater improvement of behavioral deficits and tyrosine hydroxylase expression compared to bulk LD treatment. Preadministration with LD crystalsomes resulted in notable enhancement of locomotor activities and climbing times in a PD mouse model (Li et al. 2019a). On the other hand, there may be a general issue with the MPTP model, as it is model with direct toxic lesions of DA neurons, and hence suitable for neuroprotective and symptomatic therapies but unsuitable for the evaluation of disease-modifying therapies. Therefore, use of this model as a model for disease modification can reduce credibility of these studies.

4.3.1.2 Monoamine Oxidase B and Catechol-*O*-Methyltransferase Inhibitors

Selegiline (SLG) is a monoamine oxidase (MAO) B inhibitor used for the treatment of PD, AD, and depression. An in vitro two-phase drug release profile characterized by initial burst release followed by slow release over an extended period of time (approximately 10 h) was observed for SLG nanospheres prepared using gelatin and was suitable for the oral delivery of the drug (Al-Dhubiab 2013). From SLG hydrochloride-loaded CS NPs with mean size 303.39 ± 2.01 nm, zeta potential +32.50 mV, and EE 86.20 ± 1.38%, about 82.5% of the drug was released in phosphate buffer saline (pH 5.5) using goat nasal mucosa within 28 h, and it was assumed that the drug release was controlled by more than one process, that is, superposition of two phenomena, diffusion-controlled and swelling-controlled release (Gulati et al. 2014). SLG-loaded thiolated CS NPs with particle size 215 ± 34.71 nm, zeta potential +17.06 mV, and EE 70 ± 2.71% reduced high immobility time in the forced swim tail suspension tests evaluating the antidepressant effect, and such nanoformulation was considered as suitable for nose-to-brain delivery of the drug (Singh et al. 2016). Promising potential of SLG-loaded CS NPs in the management of PD was reported also by Rukmangathen et al. (2018). Intranasal administration of SLG-loaded CS NPs showing >90% drug loading and steady in vitro and ex vivo drug release to rats resulted in 20- and 12-fold higher drug concentrations in the brain and plasma compared to oral SLG administration and better performance in locomotor activity, catalepsy, and stride length tests as well as pronouncedly increased DA, catalase activity, and GSH content in the brain (Sridhar et al. 2018). Intranasally administered SLG nanoemulsions (NEs) showed higher ROS scavenging efficiency compared to pure SLG and increased the levels of antioxidant enzymes (e.g., GSH, SOD), while decreasing those of thiobarbituric acid-reactive substances and reducing the DA loss compared to control, suggesting that the damage caused by free radicals was reduced and biochemical alterations arising during PD could be avoided. For SLG-loaded NE administered intranasally, an approximately two-fold DA concentration was estimated in comparison to haloperidol-treated rats (16.61 ± 3.06 ng/mL vs 8.59 ± 1.00 ng/mL) (Kumar et al. 2018a). An optimized formulation of NE loaded with SLG for direct nose-to-brain delivery for the treatment of PD was characterized by a spherical shape of NE with droplet size

61.43 ± 4.10 nm and enhanced permeation of the drug 3.7-fold as compared to the drug suspension. The behavioral activities of rats with haloperidol-induced PD were significantly improved by intranasally administered SLG NE as compared to the orally administered drug (Hassanzadeh et al. 2015). According to Baysal et al. (2013), SLG-loaded PLGA-b-PEG NPs can be a promising drug carrier for destabilizing Aβ fibrils in Alzheimer patients.

Rasagiline is an irreversible inhibitor of monoamine oxidase-B used in the monotherapy of early PD or as an adjunct therapy in more advanced cases. The intranasal (IN) administration of rasagiline-loaded CS glutamate NPs with mean particle size 151.1 ± 10.31 nm and EE 96.43 ± 4.23% led to significantly higher drug concentrations in brain than their intravenous (IV) administration or the IN application of the pure drug, and thus, this nanoformulation could be used for direct nose to brain targeting in PD therapy (Mittal et al. 2016). The optimized preparation of rasagiline mesylate-loaded solid lipid nanoparticles (SLNPs) fabricated with stearic acid as a lipid matrix with average mean particle size 169 nm, quasispherical shape, and smooth surface by microemulsion (ME) technique was described by Kunasekaran and Krishnamoorthy (2015).

Entacapone is a selective and reversible inhibitor of the enzyme catechol-*O*-methyltransferase (COMT) and used in combination with other drugs for the treatment of PD. Prasad et al. (2010) prepared the solid dispersion of entacapone with ca. six-fold higher bioavailability in comparison with drug suspension.

4.3.1.3 Agonists of Dopamine Receptors

Apomorphine (AMP), a nonselective DA agonist which activates both D_2-like and, to an order of magnitude lesser extent, D_1-like receptors and acts as an antagonist of 5-HT_2 and α-adrenergic receptors with high affinity, is also used in the treatment of PD. To eliminate uptake by the liver and enhance brain targeting, Wen et al. (2012) incorporated quantum dots (QDs) and AMP into liposomes and observed a 2.4-fold increase of drug accumulation in the brain due to the application of the liposomal formulation; furthermore, theranostic liposomes with a QD-drug hybrid proved to be suitable for in vivo bioimaging. AMP-loaded PEGylated liposomes with incorporated nonionic surfactant exhibited slower release behavior compared to the drug in an aqueous solution and greater stability in plasma than free apomorphine, and rapid and prolonged brain uptake of these liposomes after an IV bolus injection into rats was observed in vivo (Hsu et al. 2011). Sesame oil/cetyl palmitate as lipid matrices were used to prepare nanostructured lipid carriers (NLCs) for transport of apomorphine diester prodrugs, diacetyl apomorphine (DAA), and diisobutyryl apomorphine (DIA), to the brain. By the addition of polyethylene glycol (PEG) to NLCs, particles with the diameter of 250 nm were prepared, which largely accumulated in the brain, and the synergistic effect of prodrug and NLC strategies integration resulted in sustained release, slower release being observed with the longer carbon chain (DIA < DAA) (Liu et al. 2012). Diester prodrugs DAA and DIA also exhibited superior skin permeation compared to AMP when formulated into nano-

sized lipid emulsions, suggesting the suitability of these prodrugs for the transdermal delivery of AMP (Liu et al. 2011a). Using the incorporation of glyceryl monostearate (GMS) or polyethylene glycol monostearate (PMS) into SLNPs as emulsifiers, NPs with mean diameters 155 and 63 nm, respectively, were prepared, and after loading with apomorphine and oral administration at a dose of 26 mg/kg demonstrated 12- to 13-fold higher bioavailability in rats than the reference solution; also, increased drug distribution in the striatum was observed after the application of the SLNPs. Moreover, when the drug was administered from SLNs containing GMS and PMS, an increase in the total number of rotations was observed (from 20 to 94 and from 20 to 115, respectively) (Tsai et al. 2011). AMP-loaded NLCs of 370–430 nm size, SLNPs, and lipid emulsions (LEs) were compared by Hsu et al. (2010) from the aspect of brain targeting and accumulation of drugs in the brain using IV administration, and it was found that the lowest drug release was observed from LEs, while NLCs could be targeted, through certain vessels, to selected brain regions. Acoustically active perfluorocarbon nanobubbles (PNBs) with a particle size of 150–380 nm were tested for encapsulation of both AMP HCl and base forms to overcome delivery problems connected with the instability of the drug and the need for frequent injections. Both drug forms encapsulated in the PNBs were protected from degradation and had retarded and sustained release profiles. However, in contrast to AMP base showing a decreased release profile with ultrasound application, following application of 1 MHz, the AMP HCl release increased two- to four-fold compared to the nonultrasound group (Hwang et al. 2009). Nanostructured sol-gel silica-DA reservoirs tested for controlled drug release in the CNS showed two regimes of release, fast and sustained DA delivery up to 24 h, and constant delivery afterward. The in vivo evaluation of such reservoirs demonstrated that the rotational asymmetry induced by AMP was reversed by intrastriatal silica-DA implants in hemiparkinsonian rats, and no motor abnormalities were observed in animals implanted with silica or silica-DA (Lopez et al. 2011).

Bromocriptine (BRC), a derivative of ergoline, is a DA agonist used to treat PD. BRC-loaded CS NPs with mean size 161.3 ± 4.7 nm, zeta potential +40.3 ± 2.7 mV, loading capacity 37.8 ± 1.8%, and EE 84.2 ± 3.5% demonstrated a brain–blood ratio of 0.69 ± 0.031 at 0.5 h when administered intranasally in mice, while the IN administration of BRC solution resulted in a ratio of only 0.47 ± 0.04, and the IV administration of these NPs was considerably less effective, indicating that the direct nose-to-brain transport bypasses the BBB. Moreover, a reversal in catalepsy and akinesia behavior of animals receiving the BRC-loaded NPs was observed when compared to haloperidol-treated mice, and it was more pronounced than in the case of treatment with BRC solution (Md et al. 2013). The coefficient of permeability of BRC-loaded CS NPs (0.9997×10^{-2} cm^{-2} h^{-1}) through the nasal mucosa was higher than that of the drug solution (0.409×10^{-2} cm^{-2} h^{-1}) (Md et al. 2012). Although BRC could be encapsulated both in monoolein aqueous dispersions (MADs) and in NLCs with high EE, only NLCs provide long-term therapeutic effects probably extending BRC half-life in vivo. In a study of the effects of the used formulations on motor disabilities in an in vivo model of PD, using 6-OHDA hemilesioned rats, the BRC NLCs, similarly to free BRC, decreased the immobility

time in the behavioral bar test specific for akinesia and enhanced the number of steps in the drag test specific for akinesia/bradykinesia, the effect of BRC NLCs being longer lasting (5 h) (Esposito et al. 2012). Thongrangsalit et al. (2015) reported that a BRC tablet of a self-microemulsifying system adsorbed onto a porous carrier stimulated secretion of lipoproteins for brain cellular uptake. Although a significantly lesser amount of drug permeated from such tablets, increased drug uptake was observed. IN administered BRC-loaded CS NPs exhibited significantly higher DA concentration (20.65 ± 1.08 ng/ml) in comparison with haloperidol-treated mice (10.94 ± 2.16 ng/ml) and could markedly revert the selective degeneration of the dopaminergic neurons in haloperidol-treated mice (Md et al. 2014). BRC encapsulated in NLCs prepared using a tristearin/tricaprin mixture was released from this formulation in a prolonged way for 48 h and, similarly to free BRC, decreased the time spent on the blocks (i.e., attenuated akinesia) in the bar test, although the effect of encapsulated BRC was more rapid in onset and prolonged (Esposito et al. 2008). BRC alginate nanocomposite was effective in reducing PD symptoms in transgenic flies when mixed in the diet, which was reflected in a considerable dose-dependent delay in the loss of climbing activity and activity pattern after the treatment with 0.5, 1.0, and 1.5 μM BRC nanocomposite over 24 days (Siddique et al. 2016).

Pramipexole (PMX) is a DA agonist of the nonergoline class used to treat PD and restless legs syndrome. IN administration of PMX-loaded CS NPs with particle size of 292.5 nm and EE 91.25% to PD rats resulted in reduced motor deficit in the form of catalepsy compared to drug nasal solution or oral marketed tablets, as well as increased SOD and catalase activities and pronouncedly increased DA level in the brain (Raj et al. 2018). In CS NPs loaded with PMX hydrochloride (Prami®), intensive interactions between the drug and the CS matrix were observed, and the NPs exhibited mucoadhesive properties, which decreased with increasing drug content, and demonstrated sustained in vitro drug release in simulated intestinal fluid, indicating that they could be further investigated for the controlled oral delivery of Prami (Papadimitriou et al. 2008).

Ropinirole is a DA agonist of the nonergoline class used to treat PD and restless legs syndrome. It was demonstrated that the composition of spheroid polymer-lipid MPs loaded with ropinirole hydrochloride (RH) with the size ranging from 2.09 to 2.41 μm can partly modulate the drug release, and a 235-fold enhancement of RH permeation was observed compared to the control in ex vivo studies conducted across sheep nasal mucosa when the drug was co-formulated with trimethyl-CS of low molecular weight; thus, this formulation could become a promising carrier for the nasal delivery of RH (Karavasili et al. 2016). CS-coated oil-in-water NEs administered intranasally in haloperidol-induced PD rat models exhibited a rather high mucoadhesive potential, deep localization in the brain, and significantly high area under the curve ($AUC_{0 \rightarrow 24h}$) and amplified C_{max} in Wistar rat brain and plasma over the IV treatment group, whereby they were the most effective in DA recovery in rats (Mustafa et al. 2015). RH-loaded CS NPs prepared by an ionic gelation method were characterized by sustained release profiles for up to 18 h, and their IN administration caused a significantly higher RH concentration in the brain of rats and a

higher brain–blood ratio at 0.5 h (0.386 ± 0.57) than the IN application of RH solution (0.251 ± 0.09) indicating direct nose-to-brain transport, bypassing the BBB (Jafarieh et al. 2015). Polysorbate 80-coated RH-loaded CS NPs were stable over 3-month storage, showed initial burst drug release followed by a sustained release over 10 h in vitro, while in vivo after 1 h of dose administration, they displayed higher drug concentration in the brains of Wistar rats and lower drug accumulations in liver, spleen, and kidney than the uncoated RH-loaded CS NPs or pure RH (Ray et al. 2018). Investigating the effect of homogenization on the fate of ropinirole-loaded true NE in brain translocation, Mustafa et al. (2012a) found that the formulation must be directly transported from the nasal cavity into the cerebrospinal fluid (CSF), whereby the homogenization effect radically improved the brain uptake of the drug. A CS-coated intranasal ropinirole NE used for the better management option of PD demonstrating high drug translocation in different parts of Wister rat brain in the ex vivo investigation was described by Mustafa et al. (2012b). Gabal et al. (2014) investigated the effect of surface charge on the brain delivery of optimized NLCs with a size of <200 nm and the absolute zeta potential value of approximately 34 mV incorporated in poloxamer in situ gels loaded with RH and administered intranasally and found that the absolute bioavailability of the drug loaded anionic and cationic NLCs in situ gels was higher than that of the IN administered drug solution. The anionic NLC in situ gel provided nearly 1.2-fold higher drug-targeting efficiency in the brain (158.5%) than the cationic NLC in situ gel. Amphiphilic triblock copolymers of poly(propylene succinate) (PPSu) and PEG were used for the preparation of core-shell NPs with hydrophobic PPSu and hydrophilic PEG forming the core and shell and loaded with a hydrophilic (ropinirole) or a hydrophobic (tibolone) drug. These NPs with mean particle size 150–300 nm released hydrophilic ropinirole at a much higher rate than hydrophobic tibolone, indicating that these copolymers can be useful especially for controlled drug delivery involving relatively hydrophobic drugs (Vassiliou et al. 2010). A ME of RH with globule size 160.2 ± 3.87 nm and zeta potential −4.24 mV that was investigated for transdermal application enhanced the permeation of the drug across the rat skin and the porcine ear skin 3.5- and 2-fold, respectively, compared to the hydrogel, counteracted catalepsy in the haloperidol-induced catalepsy rat model ten-fold better as compared to the marketed tablets, and ameliorated the motor function in the rotenone-induced Parkinsonism rat model by 76%, while the application of the oral tablet resulted in only 5% restoration of the normal function (Patel et al. 2014). The formulation of ethosomal gel prepared by incorporation of optimized ethosomal suspension into gel base and loaded with RH delivered the drug into the systemic circulation by the transdermal route in amounts equal to those delivered at peroral administration, however, at the rate slow enough to achieve longer required blood levels (Mishra et al. 2013). A NE gel increased the ropinirole skin permeation rate 7.5-fold as compared to the conventional hydrogel (Azeem et al. 2009). Azeem et al. (2012) tested the transdermal ropinirole-loaded NE gel in rats with 6-OHDA-induced Parkinsonism and found more extended drug release from this gel than from a conventional gel and orally administered tablet Ropitor®, the relative bioavailability of ropinirole being enhanced more than two-fold. While in an aqueous

solution with or without a penetration enhancer, the hydrophilic drug RH could not be transported across rat skin over the course of 12 h of application, NE formulations used as a carrier vehicle significantly increased the permeation rate of the drug across the rat skin from 0 to 63.23 μg/cm^2/h, and the lag time was reduced from more than 12 h to about 2.7~4.0 h. In addition, the physicochemical stability of drug-loaded NE formulation after 3-month storage at 25 °C was proven (Tsai et al. 2014).

Piribedil (PBD) is a piperazine derivative which acts as a D_2 and D_3 receptor agonist and also α_2-adrenergic antagonist and is used to treat PD. Peroral administration of suspensions of PBD-loaded solid lipid MPs and NPs showing a controlled release rate in rabbits led to higher drug bioavailability as compared to the pure drug (Demirel et al. 2001). The investigation of the effect of randomly methylated β-cyclodextrin (RAMEBCD) on the transdermal diffusion of PBD and S-9977 hydrochloride, a novel cognition enhancing drug, through hairless rat skin demonstrated that it decreased the transdermal flux of PBD due to the formation of an inclusion complex, but increased the percutaneous absorption of S-9977 hydrochloride two-fold, and, using a combination of oleic acid and RAMEBCD, the flux of S-9977 hydrochloride can be enhanced approximately 30-fold (Legendre et al. 1995). MPs loaded with PBD and composed of two distinct compartments, one containing PLGA and another composed of acetal-modified dextran and PLGA, were investigated for drug delivery into cochlear fluids. Seven days post infusion, PBD was detectable in the cochlear fluids, and the segmented MPs were relatively inert, persisted, released their contents and were functionally and biologically compatible with cochlear function (Ross et al. 2016).

Rotigotine (RGT) is a DA agonist of the nonergoline class used to treat PD and restless legs syndrome. RGT-loaded CS NPs for nose-to-brain delivery prepared by the ionic gelation method with hydrodynamic particle size of 75.37 ± 3.37 nm, small polydispersity index (PDI) 0.368 ± 0.02, zeta potential of 25.53 ± 0.45 mV, and EE 96.08 ± 0.01 showed the overall improvement ratio for flux and permeability coefficient compared with RGT solution (4.88 vs. 2.67) (Tzeyung et al. 2019). Lactoferrin (Lf)-modified RGT NPs applied for nose-to-brain delivery in the rat 6-OHDA model of PD delivered greater sustained amount of the drug to brain and showed more effective targeting to the striatum than drug NPs with unmodified surface and pronouncedly attenuated nigrostriatal dopaminergic neurodegeneration (Yan et al. 2018). Neuroprotective effects of CeO_2 NPs in 6-OHDA-induced Parkinsonian rats through their antioxidant and antiapoptotic effects reflected in partial neuroprotection against disturbances in motor performance and partially decreased apoptosis and oxidative stress in preventive group were described by Hegazy et al. (2017a). By addition of 1% Carbomer 1342 to the ME containing 68% water, 6.8% Labrafil®, 13.44% Cremophor® RH40, 6.72% Labrasol®, and 5.04% Transcutol® HP, a ME-based hydrogel for the transdermal delivery of RGT with lower application site reactions was prepared by Wang et al. (2015). The hydrogel demonstrated 105.76% ± 20.52% bioavailability as compared to the marketed RGT patch Neupro® and less skin irritation. RGT flexible liposome formulations stimu-

lating drug absorption and displaying a 34% higher skin penetration rate than that of pure RGT were patented by Liu et al. (2009).

Amantadine (AA) is a drug that is approved by the US Food and Drug Administration to be used as both an antiviral and an antiparkinsonian drug. According to Wu et al. (2014), AA can be associated with zwitterionic phosphatidylcholine (PC) bilayers but has an insignificant effect on the flip-flop behavior of PC molecules, except at high concentrations. On the other hand, in negatively charged dipalmitoylphosphatidylglycerol (DPPG), a low concentration of AA (e.g., 0.20 mM) in the subphase instantly disturbed the outer lipid leaflet; however, subsequently, the outer leaflet returned to the original orderly packed state, while the application of a higher concentration (e.g., 5.0 mM) led to the immediate disorder of the packing state of the outer lipid leaflet. Using EPR spectroscopy, it was ascertained that spin-labeled amantadine (AA-SL) can penetrate into the gel-phase membrane of multilamellar liposomes composed of L-α-dimyristoyl-, L-α-dipalmitoyl-, and L-α-distearoylphosphatidylcholine with the same partitioning as it penetrates into the fluid-phase membrane, and at least a part of the AA-SL molecule is deeply buried in the hydrocarbon chain region of the membrane (Subczynski et al. 1998). AA-based ion pair amphiphiles prepared using oleic acid surfactant, which can self-assemble into vesicles of 200–300 nm size, in aqueous solution loaded into PLGA-PEG-PLGA copolymer hydrogel, are a suitable drug delivery system with a long-term controlled drug release profile (Yang et al. 2014a, b).

4.3.2 Antiparkinson Experimental Nanoformulations

Cerebrolysin (CBL) is a mixture of peptides purified from pig brains, including brain-derived neurotrophic factor, glial cell line-derived neurotrophic factor, nerve growth factor, and ciliary neurotrophic factor (Menon et al. 2012). The IV administration of CBL-loaded TiO_2 nanospheres (in a dose of 3 ml/kg) in a mouse model of PD resulted in a pronounced increase in tyrosine hydroxylase (TH)-positive cells in substantia nigra pars compacta and striatum as compared to bulk CBL, and it prevented enhanced α-Syn levels in the brain and in the CSF and neuronal nitric oxide synthase in the in PD brain compared to control group and notably improved behavioral function as well. Moreover, the timed release of CBL from this nanoformulation exhibited higher neuroprotective effects in PD compared to bulk drug (Ozkizilcik et al. 2018a). Administration of TiO_2-nanowired CBL and rearing in enriched environment induced neuroprotective-neurorestorative effects resulting in morphofunctional improvement by activation of survival pathways after DA depletion in a preclinical rat model of PD (Requejo et al. 2018). The potential of PLGA NPs as a sustained release system for IV administration of CBL, a peptide mixture able to ameliorate symptoms and delay the progression of neurological disorders, such as AD and dementia, in normal and brain-injured rats was studied by Ruozi et al. (2015) who found that such formulation was able to reduce brain pathology following traumatic brain injury. Using biocompatible TiO_2 nanofibers loaded with

CBL for effective delivery of this neuroprotective agent, excellent neuroprotective effects in traumatic brain injury were observed (Ozkizilcik et al. 2018b).

Domperidone (DOM) is a peripherally selective dopamine-D_2 receptor antagonist that can be used to treat gastrointestinal symptoms in PD. The experiments with a model drug rhodamine 123 having, like DOM, low brain permeability demonstrated that amphiphilic copolymers based on the hydrophilic poly(*N*-(2-hydroxypropyl)methacrylamide (poly(HPMA)) comprising randomly distributed hydrophobic poly(laurylmethacrylate) could be considered as a promising delivery system for neurological therapeutics (Hemmelmann et al. 2012). It was also found that DOM encapsulated into these amphiphilic copolymers was able to cross the BBB and affected motor behavior in animals (Hemmelmann et al. 2011). Clemens-Hemmelmann et al. (2016) found that 10 min after the intraperitoneal injection of DOM noncovalently absorbed into micellar aggregates of poly(HPMA), which was copolymerized with hydrophobic lauryl methacrylate (10 mol%), the drug was detected in the blood and brain of mice, and the highest serum and brain DOM levels representing a 48-fold increase in serum, in contrast to mice injected with the bare drug, were estimated 40 min after injection. DOM encapsulated into amphiphilic poly(HPMA)-co-poly(laurylmethacrylate) copolymer aggregates was able to cross the BBB and affected motor behavior in animals. Spherical DOM-loaded SLNPs and NLCs fabricated by hot homogenization with subsequent ultrasonication using trimyristin as solid lipid, cetyl ricinoleate as liquid lipid, and a mixture of soy phosphatidylcholine (99%) and Tween-80 as a surfactant and having the particle size of about 30 nm and EE 87.84% and 90.49%, respectively, showed controlled release over a period of 24 h (Thatipamula et al. 2011). The transdermal application of the NE formulation containing oleic acid (4% w/w), polysorbate 20 (10% w/w), diethylene glycol monoethyl ether (20% w/w) and water (64% w/w) with small droplet size (<90 nm), uniform size distribution, and low viscosity (<160 mP) loaded with DOM had 3.5-fold higher relative drug bioavailability than the oral drug suspension, and the effective drug plasma concentration was maintained for 16 h after the transdermal application, indicating that the formulation can be used for transdermal DOM delivery for a prolonged period of time (Akhter et al. 2008).

Citicoline (CCL) is a psychostimulant/nootropic that can be considered as a valuable coadjuvant for treating cognitive impairment in chronic degenerative CNS diseases such as AD, PD-associated dementia, and ischemic stroke (e.g., Eberhardt et al. 1990; Milani 2013). The encapsulation of CCL in Tf-coupled liposomes significantly improved the radioprotective effect—approximately eight-fold in the epithelial ovarian cancer cell line OVCAR-3 and two-fold in human umbilical vein endothelial cells (HUVEC)—as compared to the free drug; this effect could be explained via the entry of CCL into cells through Tf receptor-mediated endocytosis (Reddy et al. 2006). A CCL sodium liposome solid preparation useful for protecting the brain and neurons and preventing and/or treating diseases was patented by Liao (2011); a sustained release liposomal injection used for improving brain uptake efficiency, containing CCL entrapped in ammonium sulfate liposomes, was patented by Misra et al. (2010).

Nanomicellar complex of carnosine and α-lipoic acid administered intraperitoneally to Wistar rats after receiving a single MPTP dose intranasally were able to normalize the total antioxidant activity in the brain tissue and metabolism of DA and serotonin (5-hydroxytryptamine, 5-HT) and effectively restored the level of DA metabolites, and the level of 5-HT metabolite homovanillic acid was restored to the values observed in the intact animals (Kulikova et al. 2018).

Optimized liposomes loaded with pyrazoloanthrone, a c-Jun-*N*-terminal kinase (JNK-3) inhibitor that could stop or retard the rate of apoptosis of neuronal cells, with a mean size of 112.33 ± 0.84 nm, a zeta potential of −19.40 mV, and 78.96 ± 0.28% drug EE exhibited sustained release of pyrazoloanthrone up to 24 h, and treated Wistar rats showed 4.82-fold greater pyrazoloanthrone $AUC_{(0 \rightarrow 12h)}$ over the period of 12 h compared with drug suspension (Ambhore et al. 2017).

For localized delivery of curcumin (CUR), a diarylheptanoid, belonging to the group of curcuminoids, into brain, Zhang et al. (2018a) used CUR-polysorbate 80-modified cerasomes (110 nm) combined with ultrasound-targeted microbubble destruction, whereby efficient CUR delivery to the striatum of MPTP-induced PD mice was achieved, resulting in α-Syn removal and considerably improved behavioral phenotype and the treatment ameliorated the DA depletion during 2-week postobservation after combined treatment with a dose 15 mg CUR/kg and ultrasound-targeted microbubble destruction.

PEGylated PLGA NPs used as a smart delivery carrier of selective MAO B inhibitor (coumarin C75, IC_{50} = 28.89 ± 1.18 nM) with sizes approx. 105 nm and zeta potential of −10.1 mV and EE approx. 50% were able to achieve 27,828-fold higher final C75 concentration in the nano formulation (807 ± 30 μM) than its IC_{50} value in vivo, but they were not cytotoxic (Fernandes et al. 2018).

A reinforced cross-linked composite polymeric system designed as a prolonged-release device for the site-specific delivery of nicotine with the potential for intrastriatal implantation in PD interventions consisted of nicotine-loaded electrolyte-cross-linked alginate-hydroxyethylcellulose gelispheres compressed within an external polymeric matrix, comprising hydroxypropyl methylcellulose (HPMC), polyethylene oxide (PEO), or PLGA. The PLGA matrix degradation was insignificant; the release of nicotine from the compressed polymeric matrices was retarded over several days, and at exposure to simulated CSF, zero-order release for 50 days was observed (Kumar et al. 2018b).

Fe_3O_4-modified resveratrol (RES) liposomes (RES-lips@Fe_3O_4) showing good stability exhibited sustained and slow drug release in vitro and were able to cross the BBB, resulting in increased drug amount at the target site under the external magnetic field (Wang et al. 2018a). Treatment with nanoscale RES more efficiently mitigated the rotenone-induced Parkinson's like behavioral alterations, biochemical and histological changes, oxidative stress, and mitochondrial dysfunction in rats than application of bulk RES (Palle and Neerati 2018). The benefits and limitations of RES, a natural polyphenolic nonflavonoid compound with strong antioxidative and anti-inflammatory activity in the treatment of neurological diseases using different nanotechnology strategies, were summarized by Andrade et al. (2018).

Electromagnetized AuNPs in the presence of specific electromagnetic field conditions could facilitate an efficient direct lineage reprogramming to induced DA neurons in vitro and in vivo. Thus, symptoms in in vivo mouse PD models could be attenuated efficiently and noninvasively by stimulation of AuNPs using electromagnetic field resulting in dopaminergic neuron reprogramming (Yoo et al. 2017). Au nanoclusters protected with *N*-isobutyryl-L-cysteine successfully prevented α-Syn fibrillation in vitro and exhibited superior neuroprotective effects in PD cell models and pronouncedly reversed dopaminergic neuron loss in substantia nigra and striatum of sick mice, which was reflected in significantly ameliorated behavioral disorders of sick mice (Gao et al. 2019). Based on a computational analysis focused on the α-Syn activity with biocompatible metal NPs, such as AuNPs and superparamagnetic iron oxide NPs (SPIONs) and CeO_2 NPs, it was found that CeO_2 NPs showed the best fit in the active site α-Syn with good contacts and interaction and could be considered as potential inhibitor of α-Syn, and thus, they could be applied as nanoscale drug against the PD (Kaushik et al. 2018). In addition, CeO_2 NPs were found to improve motor dysfunctions induced by 6-OHDA injection in rats and a dose of 1 mg/kg of CeO_2 NPs partially ameliorated striatal DA and rescued apoptosis without notable effect on oxidative stress (Hegazy et al. 2017b).

AgNPs prepared using *Mucuna pruriens* L. seed extract showing several shapes (e.g., rectangle, oval, and spherical) and the mean particle diameter of 36.5 nm applied at doses of 5, 15, and 20 mg/kg body weight pronouncedly lowered the catalepsy symptoms in mice, best results being observed with a dose of 5 mg/kg body weight (Sardjono et al. 2018).

Cu_xO nanoclusters with a mean size of 65 ± 7 nm prepared using phenylalanine as the structure-directing agent which could mimick the activities of peroxidase, SOD, catalase, and GSH peroxidase and eliminate ROS were found to inhibit neurotoxicity in a cellular model of PD reflected in improving memory of PD mice (Hao et al. 2019).

Investigation of CeO_2 NPs systems for selective scavenging of mitochondrial, intracellular, and extracellular ROS in PD showed that CeO_2 NPs inhibited the microglial activation and lipid peroxidation and protected the TH in the striata of PD model mice (Kwon et al. 2018). The overview related to CeO_2 NPs use in neurodegenerative disease and hypothesized mechanism of their action was presented by Rzigalinski et al. (2017).

A dose of 3.25 mg/kg SWCNTs dual modified by PEG and Lf and loaded with DA (3.25 mg/kg) attenuated the oxidative stress and inflammatory responses in parkinsonian mice and increased the TH-immunoreactive density in the striatum (Guo et al. 2017a).

You et al. (2018a) designed a polymeric NP system modified with brain-targeting peptide rabies virus glycoprotein (RVG) 29 that can deliver the iron chelator deferoxamine intracerebrally. This considerably reduced Fe content and oxidative stress levels in the substantia nigra and striatum of PD mice, as well as dopaminergic neuron damage and also reversed neurobehavioral deficits of PD mice. A negative impact on the brain or other organs was not observed.

Plasmid NPs encoding human glial cell line-derived neurotrophic factor (GDNF) administered intranasally 1 week before a unilateral 6-OHDA lesion in a rat model of PD induced transgene expression in the brain and protected DA neurons in a model of PD. Three to four weeks after the lesion they were able to reduce amphetamine-induced rotational behavior and notably preserve the dopaminergic fiber density and cell counts in the lesioned substantia nigra and nerve terminal density in the lesioned striatum, providing improved neuroprotection compared to the naked plasmid (Aly et al. 2019). Intranasal administration of a nanoformulation of GDNF encapsulated into CS-coated NLCs, the surface of which was modified with transactivator of transcription (TAT) peptide to a MPTP mouse model of PD caused motor recovery and acted as a modulator on microglia activation (Hernando et al. 2018).

Mesenchymal stem cell (MSC)-derived exosomes, which were found to be able to act as biological NPs with favorable effects in different pathological conditions including PD, show potential to be used in the therapy of PD (Vilaca-Faria et al. 2019). Dextran-coated iron oxide NPs promoted the migration capacity of human mesenchymal stem cells (hMSCs) toward lesioned DA neurons and induced the differentiation of hMSCs into DA-like neurons at the diseased sites in a mouse model of PD induced by a local injection of 6-OHDA (Chung et al. 2018). However, it is important to note that iron participates in Fenton's reaction resulting in ROS production. The release of iron ions from iron oxide NPs may result in iron accumulation in the targeted tissue, which could finally result in aggregation of proteins, including Aβ and α-Syn, in neuronal cells. Therefore, the application of iron oxide NPs in the CNS due to their properties should be thoroughly considered (Yarjanli et al. 2017).

Neutral (zwitterionic) nanoliposomes supplemented with cholesterol and decorated with PEG reduced α-Syn and ROS levels but did not affect intracellular calcium in neuronal cells, suggesting their potential to be used at treatment of PD or as a vehicle for drug delivery (Aliakbari et al. 2018). The overexpression of α-Syn resulting in the death of dopaminergic neurons could be avoided by suppressing α-Syn overexpression through RNA interference. Anionic liposomes loaded with small-interfering ribonucleic acid (siRNA)-protamine complex for α-Syn gene silencing and decorated with a RVG-derived peptide as a targeting agent displayed suitable characteristics related to delivery of the functional siRNA to mouse primary hippocampal and cortical neurons and induced α-Syn gene silencing in primary neurons without altering cell viability (Schlich et al. 2017). Bhak et al. (2018) investigated an oligomeric species of α-Syn capable of exhibiting the unit-assembly process leading to accelerated amyloid fibril formation. The spherical metastable α-Syn oligomers with a diameter about 100 Å showing mostly disordered structure are unable to seed the fibrillation; however, their shape altered drastically within the temperature range 37–43 °C, suggesting that the oligomers represent critical intermediate for the oligomeric diversification and multiple fibrillation processes and could be considered as a principal target to control the amyloidogenesis and its pathogenesis.

4.4 Anti-Alzheimer's Drugs

As mentioned above, AD represents the most common form of dementia. It is a complex and progressive neurodegenerative disorder primarily characterized by memory deterioration and a range of other cognitive deficits, which are commonly accompanied by multiple neuropsychiatric symptoms. The number of people affected by AD worldwide is expected to double to more than 100 million people by the year of 2050. Drugs approved by for the treatment of AD, such as donepezil, rivastigmine, and galantamine, belong to the cholinesterase inhibitors group and memantine belongs to the class of *N*-methyl-D-aspartate (NMDA) receptor antagonists (Bajic et al. 2016). The latest findings related to novel formulations loaded with various anti-Alzheimer agents (e.g., galantamine, deferoxamine, tacrine, tarenflurbil, rivastigmine, risperidone, CUR, quercetin, piperine, or insulin) and nanoscale delivery systems were published by Agrawal et al. (2018).

The progression of AD is accompanied by disturbances of the endosome/lysosome (EL) system and associated with accumulation of the Aβ peptides in EL vesicles of affected neurons (Kanazirska et al. 2012). The function and action of arginine metabolizing enzymes with respect to the formation of senile plaques and amyloid peptide aggregation are discussed in the review paper of Whiteley (2014) (Fig. 4.2). The possibility to use hydrophobic plant antioxidants for the prevention of amyloid transformation of proteins and other neurodegenerative processes was presented by Muronetz et al. (2014) who also analyzed the role of misfolded proteins in the regulation of the chaperone system involved in the genesis of amyloid neurodegenerative diseases (AD and prion diseases) and the role of the modification of the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase in the inhibition of glycolysis and causing apoptosis of nerve cells. Recent findings related to the use of light energy to inhibit the self-assembly process of Aβ peptides via the generation of oxidative stress by photosensitizers (e.g., natural or synthetic dyes, light-responsive nanomaterials, and photoelectrochemical platforms) were reviewed by Lee et al. (2019). A review paper focused on mitochondrial dysfunction and its role in late onset AD pathology, emphasizing recent antioxidant and enzymatic treatments used to alleviate mitochondrial dysfunction as well as current applications of targeted mitochondrial delivery in the treatment of AD, was presented by Giau et al. (2018).

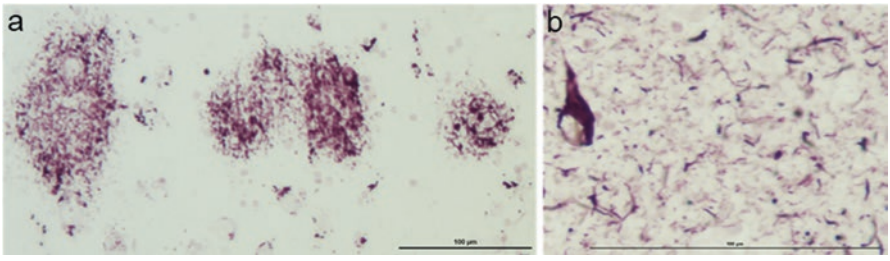

Fig. 4.2 Images of amyloid plaques (**a**) and neurofibrillary tangle (**b**)

The various targeting strategies to deliver drugs into the brain for treating various neurological diseases were also discussed by Wilson (2019). Nanotechnologies may be a promising contribution to drug delivery strategies in the future, in particular, drug carrier nano- or microsystems, which can limit side effects of drugs, including anti-Alzheimer drugs (Di Stefano et al. 2011; Ahmad et al. 2014; Burkhart et al. 2014). A review of current strategies for delivering AD drugs across the BBB focused on most promising drug delivery systems, such as polymeric NPs, liposomes, metallic NPs, and cyclodextrins (CDs), was presented by Goyal et al. (2014), Karthivashan et al. (2018), and Wong et al. (2019), while Ruozi et al. (2014) reviewed the most outstanding nanomedicine-driven approaches in AD imaging/detection and treatments. Current progress in nanodelivery systems for treating neuropsychiatric diseases such as AD and schizophrenia were presented by Silva et al. (2013).

Recent trends in analytical approaches for detecting neurotransmitters in AD were summarized by Sangubotla and Kim (2018). Detection and the quantification of amyloid aggregate surfaces which were functionalized by various magnetic moieties, such as peptides, antibody fragments, or whole antibodies of magnetic NPs, and the use of magnetic NPs for magnetic-resonance-based amyloid imaging in biomedical fields were overviewed by Pansieri et al. (2018).

In a review paper, Ross et al. (2018) focused on the progress of liposomal approaches in AD therapy, including targeted nanoliposomes using modifications with brain-penetrating peptides, Aβ-targeting ligands, or a retro-inverted peptide that inhibits Aβ aggregation and encapsulated therapeutics which efficiently inhibit Aβ and/or tau aggregation. According to Ma et al. (2018), the benefits of nanoscale anti-AD drug formulations compared to conventional drugs are as follows: i) suppression of many adverse and unfavorable pharmaceutical properties of conventional anti-AD drugs; ii) efficient production of high-titer anti-Aβ antibodies following controlled release of antigens; iii) preferential binding of some apolipoprotein-based nanoscale drugs to Aβ and increased elimination of Aβ; autophagy induced by nanoscale drugs could be facilitated to increase the elimination of Aβ or tau; nanoscale drug-induced inhibition of tau aggregation. Study of curvature effect of spherical NPs and cylindrical nanorods (NRs) on the adsorption and fibrillation of amyloid peptide chains using coarse-grained Monte Carlo simulations showed a nonmonotonic tendency in the number of assembled peptide chains with increasing size of NPs/NRs; however, due to the lower curvature, more peptides were adsorbed by NRs compared to the NPs (Li et al. 2019b).

4.4.1 Drugs Approved for Alzheimer's Disease

4.4.1.1 Competitive Cholinesterase Inhibitors

Rivastigmine (RVT) is a cholinergic agent for treating mild-to-moderate dementia of the Alzheimer's type and dementia due to PD. RVT-loaded human serum albumin (HSA) NPs designed using glutaraldehyde as a cross-linking agent and coated with

polysorbate-80 to facilitate brain targeting via endocytosis, in which the drug was completely entrapped in HSA NPs, showed 55.59 ± 3.80% release of the drug from the NPs in vitro in 12 h, indicating that the HSA NPs used as a carrier could ensure sustained delivery of RVT (Avachat et al. 2014). The IV administration of L-lactide-depsipeptide polymeric NPs loaded with RVT for targeting the drug to the brain with mean particle size 142.2 ± 21.3 nm and drug EE 60.72 ± 3.72% led to a 5.45-fold and 2-fold increase in the brain concentration of the drug as compared to plain RVT solution administered by the oral and IV routes, respectively (Pagar et al. 2014). RVT-loaded Lf conjugates with polyamidoamine generation 3.0 (PAMAM G3.0) dendrimers of 216.13 ± 2.3 nm showed sustained release up to >100 h, five-fold lesser ex vivo hemotoxicity, and 7.87-fold higher bioavailability compared to free drug and administration of this conjugate resulted in eight-fold higher drug uptake by brain after 4 h of the administration compared to free drug, which was reflected in pronounced enhancement of the overall locomotor activity over the pure drug and memory improvement (Gothwal et al. 2018).

The brain concentration after the IN administration of RVT-loaded CS NPs was significantly higher (966 ± 20.66 ng/ml; t_{max} 60 min) than after the IV administration of RVT solution (387 ± 29.51 ng/ml; t_{max} 30 min) or the IN administration of RVT hydrogen tartrate solution (508.66 ± 22.50 ng/ml; t_{max} 60 min), and the drug transport efficiency was 355 ± 13.52%, the direct transport percentage being 71.80 ± 6.71% (Fazil et al. 2012a). RVT liposomes and especially cell-penetrating peptide-modified liposomes having very mild nasal toxicity enhanced the permeability of RVT across the BBB in a murine brain microvascular endothelial cells model in vitro and after IN administration, simultaneously decreasing the hepatic first pass metabolism and gastrointestinal adverse effects (Yang et al. 2013). Ismail et al. (2013) reported the strong therapeutic effect of RVT liposomes, which nearly prevented amyloid plaque formation in the brain of $AlCl_3$-treated rats, indicating that such liposomes could be used as a potential drug-delivery system for ameliorating AD. Arumugam et al. (2008) investigated RVT liposomes formulated by the lipid layer hydration method using cholesterol and soya lecithin as lipid components for the delivery of the drug into the brain through the intranasal route, and they found that as the result of the IN administration of such liposomes, the level of the drug was significantly higher and its half-life in the brain was longer than those of the intranasally or orally administered free drug. RVT-loaded NPs of biodegradable polymers, PLGA, and poly-butylcyanoacrylate (PBCA) with particle size and drug EE 135.6 ± 4.2 nm and 74.46 ± 0.76%, respectively, for PLGA NPs and 146.8 ± 2.6 nm and 57.32 ± 0.91%, respectively, for PBCA NPs exhibiting 30.86 ± 2.07% and 43.59 ± 3.80% drug release from PLGA and PBCA NPs, respectively, in 72 h caused faster regain of memory loss in amnesic mice than RVT solution, indicating the higher extent of RVT transport into the mice brain (Joshi et al. 2010). The brain concentration of IV injected RVT can be enhanced more than 3.82-fold by binding NPs coated with 1% nonionic surfactant polysorbate 80 to PBCA (Wilson et al. 2008a). NLCs prepared using glyceryl monosterate, Capmul MCM C8, lecithin, and Tween 80 with average particle size 123.2 ± 2.3 nm and RVT EE 68.34 ± 3.4% incorporated into an in situ gelling system using 0.8% gellan gum and 15% Lutrol F 127 demonstrated a two-fold increase in nasal permeation of

the drug and a three-fold increase in enzyme inhibition effect as compared to plain RVT solution (Wavikar and Vavia 2015). Shah et al. (2015) used the quality by design approach for intranasal delivery of RVT-loaded SLNPs formulated by the homogenization and ultrasonication method using Compritol 888 ATO as lipid, Tween-80 as a surfactant, and Poloxamer-188 as a stabilizer. The prepared RVT-SLNPs were characterized by particle size 82.5 ± 4.07 nm and EE 66.84 ± 2.49%, and it was proven that the drug was incorporated into the imperfect crystal lattice of Compritol 888 ATO. Higher in vitro and ex vivo drug diffusion in comparison with drug solution and intact nasal mucosa observed after treatment with RVT-SLNPs suggested the safety of this nanoformulation for IN administration. Scialabba et al. (2012) described amphiphilic polyaspartamide copolymer-based micelles with a nanometric hydrodynamic diameter, narrow size distribution, and negative surface charge, which incorporated a large amount of RVT, for the delivery of the drug to neuronal cells, and the stability of RVT in the system after incubation in human plasma was maintained. The micelles were internalized by neuroblastoma cell lines with drug uptake depending on the micelles concentration, and no cytotoxic effect of either empty or loaded micelles on the mouse neuroblastoma cells (Neuro2a) was observed in the in vitro biological assay. RVT hydrogen tartrate-loaded mesoporous SiO_2 NPs with particle size 145 ± 0.4 nm and the zeta potential of approx. 37.6 ± 1.4 mV, respectively, were less hemolytic and caused higher inhibition of AChE compared to the pure drug (80 ± 4.98% vs 62 ± 3.25%), and brain delivery of RTG in Wistar rats was enhanced almost 127-fold in vivo (Pandey et al. 2018). RVT and five other amine-containing drugs (5-fluorouracil, chlorhexidine, nystatin, dapsone, doxazosin) successfully intercalated sodium montmorillonite (MMT), a clay with large surface area and high capacity for cation exchange, which could be used in the development of more efficient drug delivery nanosystems (Bello et al. 2015).

Galantamine (GAL) is intended for the treatment of mild-to-moderate AD and different other memory impairments, in particular those of vascular origin. The efficiency of AChE inhibition by galantamine hydrobromide (GALH), especially GALH loaded in flexible liposomes, which could readily transport GALH into brain tissues, was greatly enhanced by IN administration as compared to oral administration, suggesting some promise for this approach in brain drug targeting in AD treatment (Li et al. 2012). Mufamadi et al. (2013) designed spherical ligand-functionalized nanoliposomes for effective delivery of GAL into PC12 neuronal cells, in which GAL and the peptide ligand were incorporated into the inner core and surface of the nanoliposomes, respectively, and such optimized formulation was found to have sustained drug release with 30% of drug released within 48 h, and high drug accumulation in PC12 neuronal cells was influenced by the postengineering of peptides on the surface of the GAL-loaded nanoliposomes. In vitro drug release from GALH-loaded spherical SLNPs with size <100 nm and maximum drug EE 83.42 ± 0.63% was >90% for a period of 24 h in a controlled manner, and this nanoformulation demonstrated in vivo significant memory restoration capability in cognitive-deficit rats as compared to the native drug and offered approximately two-fold higher bioavailability than that of the plain drug (Misra et al. 2016). The complexation of GALH with CS was considered to be a promising approach to enhance the entrap-

ment of cationic drug into CS NPs, because it had inessential effect on the physicochemical properties of CS NPs. The GALH/CS complex NPs with diameter 190 nm and zeta potential +31.6 mV demonstrating prolonged drug release and improved formulation stability at 4 °C were successfully delivered to different brain regions of male Wistar rats shortly after IN administration, indicating their potential as a delivery system for anti-Alzheimer agents (Hanafy et al. 2015). Intranasal administration of GAL-loaded thiolated CS NPs was found to be pronouncedly more effective compared to oral and nasal administration of its solution, which was demonstrated by pharmacodynamic study and biochemical estimation of AChE activity in Swiss albino mice brain (Sunena and Mishra 2019). GAL-loaded polymeric NPs prepared from GAL-loaded NEs using solvent evaporation, which had hydrodynamic radius ≈20 nm, negative surface charge, stability more than 3 months, and EE >90 wt%, demonstrated a sustained drug release profile as compared to those from aqueous and micellar solutions, and the enzymatic activity of GAL was maintained at 80% after its encapsulation into the NPs (Fornaguera et al. 2015). In nanocomposites prepared by attaching GAL to ceria-containing hydroxyapatite (GAL@Ce-HAp), which were intraperitoneally injected into ovariectomized AD albino-rats, the existence of dispersed negatively charged rod-like particles conjugated with ceria nanodots prevailed, and these nanocomposites upregulated oxidative stress markers and ensured the total recovery of degenerated neurons in hippocampal and cerebral tissues as well as the disappearance of Aβ plaques. They showed also optimizable in vitro release for GAL and nanoceria, indicating that rod-like hydroxyapatite particles could be used for selective delivery of GAL and nanoceria to AD-affected brain areas (Wahba et al. 2016).

4.4.1.2 Noncompetitive Cholinesterase Inhibitors

Donepezil (DPZ) is a drug used in the symptomatic treatment of AD to improve cognition and behavior. In DPZ HCl-loaded nanofibers fabricated by electrospinning, in vitro drug release of the webs consisting of poly(vinyl alcohol) of low molecular weight (diameters of the fibers being in the range 100–300 nm) occurred immediately (<30 s) after immersion due to the formed expansive surface, independently of the drug content, while complete dissolution of cast films with the same compositions and commercial tablets required ≥30 min (Nagy et al. 2010). A DPZ-loaded CS nanosuspension with average particle size 150–200 nm and PDI 0.341 for direct IN administration was prepared by Bhavna et al. (2014a). DPZ suspension instilled into nostrils of rats at a concentration 0.5 mg/ml resulted in 7.2 ± 0.86 and 82.8 ± 5.42 ng/ml DPZ concentrations in brain and plasma, respectively, while for DPZ-loaded CS nanosuspension administered intranasally at the same dose, these concentrations dramatically increased to 147.54 ± 25.08 ng/ml and 183.451 ± 13.45 ng/ml, respectively, suggesting that such nanosuspension transported via the nasal pathway to the brain could be used as a delivery system for the treatment of AD. In another study, Bhavna et al. (2014b) showed that after IN administration in rats, DPZ-loaded CS NPs with particle size within 100–200 nm

and spherical shape with smooth morphology demonstrated higher drug transport efficiency (191.398%) and direct transport percentage (1834.480%) than DPZ solution. The release behavior of DPZ from PLGA NPs with Tween-80 coating on the NPs surface was characterized by a biphasic pattern, an initial burst release followed by a slower and continuous sustained release, and in vivo studies using gamma scintigraphy techniques demonstrated that IV application of these NPs resulted in a higher percentage of radioactivity per gram in the brain as compared with the drug solution, and it could be assumed that high concentrations of DPZ uptake in the brain are caused by coated NPs (Bhavna et al. 2014c). Administration of an ion-sensitive in situ gel loaded with NLC used for nose to brain delivery of DPZ, ensuring higher drug distribution in brain and lower drug concentration in plasma than the marketed formulation, caused also considerable improvement in cognitive function in rats with scopolamine-induced amnesia compared to the marketed formulation (Rajput and Butani 2018). The intra-/extracerebral level of high-density lipoprotein (HDL) likely plays a role in the pathogenesis of AD, whereby apolipoprotein A-I (apoA-I) gives protective outcomes. Therefore, Zhang et al. (2019a) fabricated DPZ-loaded apoA-I-reconstituted HDL (rHDL/Do) for Aβ-targeting clearance and AChE inhibition in AD therapy. This nanoformulation-exhibited controlled drug release mimicked the properties of natural lipoproteins, showed pronouncedly enhanced BBB penetration, modulated Aβ-induced neuronal damage, and, both in vitro and in vivo, facilitated microglial-mediated Aβ intake and degradation. In AD animal models, daily treatment rescued memory loss. The mean residence time of subcutaneous and intramuscular (IM) lipospheres prepared using glyceryl tripalmitate, compritol, and cetyl alcohol loaded with DPZ with average particle size 20.68–35.94 μm was found to be significantly longer (11.04 and 11.34 days, respectively) than that of the solution (0.53 days), which represents almost a 20-fold increase. The IM delivery of DPZ glyceryl tripalmitate lipospheres ensured depot release, allowing less frequent dosing (Yehia et al. 2012). By intercalating DPZ molecules into smectite clays (laponite XLG, saponite, and MMT), an inorganic–organic hybrid for drug delivery was prepared, in which DPZ molecules were stabilized in the interlayer space of clay via mono- or double-layer stacking. Moreover, coating the hybrid with Eudragit® E-100 using a spray dryer resulted in a great increase of the release rate within a short period of time (180 min) (Park et al. 2008).

Tacrine (TCR) is a centrally acting AChE inhibitor and indirect cholinergic agonist (parasympathomimetic) that can be used for the treatment of AD and other CNC disorders. Unfortunately, it is quite hepatotoxic; therefore, it is not used for AD treatment anymore. The characteristics of the preferential binding of TCR to acidic phospholipids based on the estimation of fluorescence polarization, penetration into lipid monolayers, and effects on the thermal phase behavior of dimyristoyl phosphatidic acid were presented by Lehtonen et al. (1996). TCR-loaded PLGA NPs (247–293 nm) showed lower IC_{50} value than free TCR in vitro and faster regain of memory in amnesic mice treated with PLGA NPs compared to free TCR, suggesting the higher extent of transport of TCR in mice brain (Joe and Kumar 2018). Wilson et al. (2010) found that TCR-loaded CS NPs coated with Polysorbate 80

slightly reduced the drug release from NPs, the release was diffusion controlled, and the preparation had optimal pharmacokinetic characteristics in a rat model. Elmizadeh et al. (2013) described the preparation and optimization of CS NPs and magnetic CS NPs with average particle size ranging from 33.64 to 74.87 nm as delivery systems for the anti-Alzheimer drug TCR, using Box–Behnken statistical design. It was found that the magnetic CS NPs significantly increased the concentration of TCR in the brain of animals after IV administration as compared to the free drug (Wilson et al. 2009). Eslami et al. (2016) investigated the compatibility of PBCA and CS NPs with different degrees of polymerization with a TCR unit using molecular dynamics simulation and discovered that the TCR molecule had higher compatibility with PBCA than with CS, and, in contrast to CS/TCR systems, the interaction between the TCR molecules and PBCA NPs became stronger with the increasing length of the polymer chain. The mean size and PDI of unloaded CS NPs prepared by ionic gelation using sodium tripolyphosphate as a cross-linking agent were not changed by the encapsulation of TCR, while zeta potential was increased to +38 mV due to the positive charge of TCR. The efficiency of TCR encapsulation into NPs was approximately 66%, and the NPs were stable in an acidic medium at 4 or 25 °C for at least 25 days (Hassani et al. 2015). Compared to the uncoated NPs and the free drug, the brain concentration of intravenously injected TCR can be raised by binding it to PBCA NPs coated with 1% nonionic surfactant polysorbate 80, while the accumulation of the drug in the liver and spleen is decreased (Wilson et al. 2008b). The study of TCR hydrochloride nasal delivery using albumin NPs carrying β-CD and two different β-CD derivatives (hydroxypropyl β-CD and sulphobutylether β-CD) revealed that the presence of the different β-CDs in the polymeric network had an effect on drug loading and could differently modulate NPs mucoadhesiveness and drug permeation behavior (Luppi et al. 2011). Liposomes designed using cholesterol and phosphatidylcholine partly enriched with α-tocopherol and/or omega-3 fatty acids with mean diameter ranging from 175 to 219 nm, PDI <0.22, slightly negative zeta potential, and excellent EE notably increased TCR permeability, which can be related to the fusion of liposomes with the cellular membrane, and the addition of α-tocopherol without omega-3 fatty acids improved the neuroprotective effect and antioxidant properties of liposomes (Corace et al. 2014).

4.4.1.3 NMDA Receptor Antagonists

Memantine (MEM) is a drug used for treating AD. It affects the glutamatergic system by blocking NMDA receptors and hence addressing the overactivation of glutamatergic systems in AD. It should be noted that glutamate toxicity is one of the mechanisms possibly involved in AD. Tests aimed at the improvement of the solubility of lipoyl-MEM, a potential anti-Alzheimer codrug, and its absorption through the gastrointestinal tract by SLNPs proved that SLNPs are not cytotoxic and can release the free codrug, suggesting their potential for application as a drug-delivery system for the brain (Laserra et al. 2015). Using MIT assay, Mittapelly et al. (2016)

found that nanocrystals of MEM-pamoic acid (MEM-PAM) salt are less cytotoxic and more tolerable than plain MEM HCl in murine fibroblast 3T3 cell line, and when administered as IM injection at three different doses in female Sprague-Dawley rats, the plasma levels were maintained until the 24th day of the study, suggesting that injectable nanocrystals could represent a therapeutic alternative for treating AD. SLNPs are able to protect drugs from chemical and enzymatic degradation, direct active compound toward the target site with substantially lower toxicity for adjacent tissues, and pass physiological barriers increasing bioavailability without resorting to high dosage forms, and thus, they could represent a suitable tool to pass the BBB and allow drugs to reach damaged areas of the CNS in patients affected by neurodegenerative pathologies, such as AD and PD (Cacciatore et al. 2016). MEM-loaded PLGA PEGylated NPs with average particle size 152.6 ± 0.5 nm and surface charge of −22.4 mV reduced Aβ plaques and the associated inflammation characteristic of AD and enhanced the benefit of decreasing memory impairment in transgenic APPswe/PS1dE9 mice (Sanchez-Lopez et al. 2018). MEM HCl-loaded casein NPs with particle sizes 148–317 nm and zeta potential of −46.4 mV and the optimized formulation with sodium tripolyphosphate/casein ratio of 1/3 demonstrated sustained release behavior for a period of 24 h in vitro (Rao et al. 2018). Kanazirska et al. (2012) reported beneficial effects of lysosome modulating and other pharmacological and nanocarrier agents on Aβ-treated cells, some of which facilitated the antiamyloid effects of MEM.

4.4.2 Nanoformulations of Experimental Anti-AD Compounds

SWCNTs successfully delivered acetylcholine (ACh) into the brain to treat experimentally induced AD with a moderate safety range by precisely controlling the doses, and it was found that SWCNTs preferentially enter lysosomes, the target organelles, and not mitochondria (Yang et al. 2010b). The direct delivery of a low dose of ACh via human serum albumin NPs improved both spatial learning and memory capability, whereas it reduced oxidative damage in mice without damage to the liver or interference with the inherent neurotransmitter generation (Fan et al. 2018a). Treatment with L-tryptophan NPs resulted in improved behavioral reactions in AD animal models using *Rattus norvegicus* rats (Miri et al. 2019).

α-Bisabolol-loaded SLNPs were found to mitigate Aβ aggregation and protect neuro-2a cells from Aβ-induced neurotoxicity (Sathya et al. 2018a). Bernardi et al. (2012) found that indomethacin-loaded lipid-core nanocapsules reduced the damage triggered by intracerebroventricular injection of $A\beta_{1\text{-}42}$ in AD models, were able to increase interleukin-10 release and decrease glial activation and c-jun N-terminal kinase phosphorylation, significantly attenuated the impairment of behavior, and suppressed glial and microglial activation. Meloxicam, a nonsteroidal anti-inflammatory drug reducing hormones that cause inflammation and pain in the body, encapsulated into polymeric nanocapsules was observed to protect against learning and memory impairments, neuronal loss, and oxidative stress in an Aβ

mouse model of AD (Ianiski et al. 2012). The ability of meloxicam-loaded nanocapsules to reverse the memory impairment induced by Aβ is associated with Na^+, K^+-ATPase (Ianiski et al. 2016). Tarenflurbil (TFB) was tested regarding its potential to treat AD, and it was proven that this compound is a potent reducer of Aβ levels, however, failed in clinical studies (Mehta et al. 2017; Panza et al. 2019). NPs prepared by loading TFB into PLGA (TFB NPs) and SLNPs (TFB SLNPs) with particle size <200 nm and drug EE 64.11 ± 2.21% and 57.81 ± 5.32%, respectively, ensured sustained drug release compared to the pure drug, with increased residence times of the drug at the target site. Because the bioavailability of TBF decreased as follows: TFB NPs (IN) > TFB SLNs (IN) > TFB solution (IN) > TFB suspension (oral), and drug-targeting efficiency and drug transport percentage of TFB NPs (287.24% and 65.18%, respectively) and TFB SLNs (183.15% and 45.41%, respectively) exceeded those of the TFB solution and suspension, it can be assumed that TFB could be transported directly to the brain via the olfactory pathway after IN administration of polymeric and lipidic NPs (Muntimadugu et al. 2016). Self-nanomicellizing solid dispersion of edaravone showed excellent cellular uptake and neuroprotective effect in SH-SY5Y695 APP cell line, was nontoxic up to 414 μM/kg dose, and was able to reverse AD-like behavior deficits of APP/PS1 mice (Parikh et al. 2018).

PLGA NPs loaded with vitamin D-binding protein inhibited Aβ aggregation in vitro and IV injection to 5XFAD mice pronouncedly mitigated the Aβ accumulation, neuroinflammation, neuronal loss, and cognitive dysfunction in treated animals, suggesting that that formulation could be considered as a promising therapeutic candidate for the treatment of AD (Jeon et al. 2019).

Methylene blue-loaded nanospheres based on PLGA and nonionic amphiphilic CD assemblies with particle size approximately 200 nm were found to provide significant neuroprotection against the metabolic effects of iodoacetic acid, especially in the presence of the reduced nicotinamide adenine dinucleotide electron donor (Cannava et al. 2016). Giglio et al. (2016) reported amino-CD oligomers as protective agents of protein aggregation. NPs conjugated to chelators that can cross the BBB, chelate metals, and exit through the BBB with their corresponding complexed metal ions could be utilized as safe and effective means for decreasing the metal load in neural tissue, thus mitigating the amount of oxidative stress and its consequences (Liu et al. 2010).

Amphiphilic liquid crystalline nanocarriers (cubosome, hexosome, spongosome, and liposome particles) suitable for the encapsulation of CUR to stop progressive neuronal loss in AD, PD, and Huntington's diseases, and ALS were summarized by Rakotoarisoa and Angelova (2018). Tiwari et al. (2014) reported that CUR-loaded PLGA NPs potently induced adult neurogenesis and reversed cognitive deficits in an AD model via the canonical Wnt/β-catenin pathway. CUR-loaded PLGA NPs, coupled with the Tet-1 peptide having affinity to neurons and possessing retrograde transport properties, were able to destroy amyloid aggregates, showed antioxidative property, and were not cytotoxic, suggesting that they could be a potential therapeutic tool against AD (Mathew et al. 2012). CUR-loaded SeNPs encapsulated in PLGA nanospheres were found to decrease the Aβ load in brain samples of AD

mice and significantly improved the memory deficiency of the model mice. Based on the enhanced therapeutic efficacy in AD lesions observed with the use of Se-PLGA-targeting delivery system to amyloid plaques in transgenic mice (5XFAD), it could be considered a valuable CUR delivery system for effective treatment of AD (Huo et al. 2019). In self-assembled nanogels of CUR-hyaluronic acid conjugates that were proved to inhibit beta fibrillogenesis and mitigate amyloid cytotoxicity more efficiently than the free drug, CUR encapsulation into nanogels protected cells from the toxicity of free CUR, the hydrogel network hindered interactions between Aβ molecules, and the counteraction of the hydrophobic binding between Aβ and the conjugated CUR against the electrostatic repulsion between similarly charged Aβ and hyaluronic acid was observed (Jiang et al. 2016). Epigallocatechin-3-gallate (EGCG)-CUR bimodified hyaluronic acid self-assembled into nanogels reduced cytotoxicity and inhibited Aβ protein aggregation more efficiently than formulations of individual antioxidants, suggesting synergism of EGCG and CUR. Thus, dual-inhibitor nanosystem is promising for the development of potent agents against AD (Jiang et al. 2018). CUR-loaded CS-bovine serum albumin NPs improved drug penetration through the BBB, promoted the activation of microglia, accelerated the phagocytosis of the Aβ, and potentially enhanced A_{42} phagocytosis through modulating macrophage polarization in AD (Yang et al. 2018a). A novel low-density lipoprotein-mimic NLC modified using a high level of Lf and loaded with CUR demonstrated effective uptake in the brain capillary endothelial cells in an AD model of rats, effectively permeated the BBB, and preferentially accumulated in the brain (2.78-fold more than the unmodified NLC) (Meng et al. 2015). Stable CUR-conjugated nanoliposomes, with CUR at the surface, downregulated the secretion of the amyloid peptide, partially reduced Aβ-induced toxicity, and were able to specifically stain Aβ deposits in vivo, indicating that they could be applied in the diagnosis and targeted drug delivery in AD (Lazar et al. 2013). The nanoformulations of polyvinylpyrrolidone-encapsulated CUR conjugate with AuNPs decorated on the surface were found to inhibit $Aβ_{1-16}$ aggregation and were also able to disintegrate already formed aggregates (Brahmkhatri et al. 2018). CUR-loaded PBCA NPs coated with polysorbate 80 with average diameter 152.0 nm and average drug loading 21.1% reached a greater AUC value and 14-fold longer residence time in plasma than the control solution, which indicates the improved transport of CUR to the brain (Sun et al. 2010). Conjugates of CUR with a zwitterionic polymer, poly(carboxybetaine methacrylate) (CUR@pCB) self-assembled into nanogels of 120–190 nm and more efficiently inhibited $Aβ_{42}$ fibrillation than free CUR, and the conjugate with 1.9 degree of substitution showing the best performance when applied at a dose 5 μM achieved similar effect than 25.5 μM of free CUR and also increased the cell viability by 43%. The dense hydration layer on the conjugates greatly stabilized the bound Aβ on CUR anchored on the polymer and suppressed the conformational transition of the protein to β-sheet-rich structures (Zhao et al. 2019).

Evaluation of the neuroprotective role of EGCG loaded NPs against $AlCl_3$-inducing neurobehavioral and pathological changes in AD rats conformed beneficial effect of EGCG NPs. This was reflected in both neuritic plaques and

neurofibrillary tangles absence in rat brains and pronounced attenuation of neurobehavioral impairments of animals (Singh et al. 2018a). Nanolipidic particles increased the bioavailability of EGCG in vivo more than two-fold as compared to pure EGCG and improved α-secretase-inducing ability; thus, they could be useful for the treatment of AD (Smith et al. 2010). EGCG NPs with particle size 300 nm showing spherical to slightly ellipsoid shape showing better antioxidant and metal chelation potential and lower cellular toxicity than free EGCG were able to strongly inhibit both $A\beta_{42}$ and Al(III)–induced $A\beta_{42}$ fibrillation in vitro due to the generation of soluble $A\beta_{42}$ amorphous aggregates instead of insoluble $A\beta_{42}$ oligomers and fibril generation (Singh et al. 2018b). Quercetin and rosmarinic acid loaded liposomes surface functionalized with phosphatidic acid and apolipoprotein E crossed the BBB and delivered the antioxidants to the brain, and they were able to lower Aβ plaque formation; thus, they could be used in the management of $A\beta_{1\text{-}42}$-induced neurotoxicity (Kuo et al. 2018). The neuroprotective effect of hesperetin and nanohesperetin manifesting as improved recognition memory and a reduction of the elevated oxygen stress in rat model of AD was described by Kheradmand et al. (2018).

Tf-modified liposomes loaded with α-mangostin, a polyphenolic xanthone that protects and improves the survival of cerebral cortical neurons against Aβ oligomer-induced toxicity in rats, were characterized by improved penetration through the BBB, indicating that such nanoformulation could serve as drug delivery system for brain targeting (Chen et al. 2016).

A review paper focused on effective RES brain delivery for neurological disorders prevention and treatment was presented by Andrade et al. (2018). In the treatment of $A\beta_{1\text{-}42}$-infused animals that display impaired learning with RES-loaded lipid-core nanocapsules, a significant increase of RES concentration in the brain tissue was achieved, and RES in the nanocapsules was able to revert the deleterious effects of $A\beta_{1\text{-}42}$, while the treatment with pure RES had only partial beneficial effects (Frozza et al. 2013a). In situ gel fabricated by incorporation of the RES NLC in gellan gum and xanthan gum showed five-fold higher permeation across the nasal mucosa and pronouncedly improved the memory function in rats with scopolamine-induced amnesia compared to RES suspension-based in situ gel (Rajput et al. 2018). SeNPs were found to improve the application of RES in AD treatment when applied as RES-functional SeNPs (RES@SeNPs), whereby this modification showed synergistic effect on Cu^{2+}-induced $A\beta_{42}$ aggregation, ROS generation and protected PC12 cells from $A\beta_{42}$-Cu^{2+} complexes-induced cell death and Res@SeNPs in long-term use could more efficiently reduce $A\beta_{42}$ toxicity than free RES (Yang et al. 2018b).

Phytol-loaded PLGA NPs showing sustained release of phytol were able to disrupt amyloid aggregates, showed anticholinesterase and antioxidative properties, and were noncytotoxic in Neuro2a cells (Sathya et al. 2018b). A biodegradable PLGA-ginsenoside Rg3 nanoformulation was reported as a new theranostic material capable of encapsulating natural nutraceuticals for the detection and treatment of AD (Aalinkeel et al. 2018). Brazilin, a red pigment obtained from the wood of the brazil wood family (*Caesalpinia* sp.) showing higher affinity for $A\beta_{42}$ than of Zn^{2+},

could sequester Zn^{2+} from the $A\beta_{42}$-Zn^{2+} complex and it was found to inhibit Zn^{2+}-mediated Aβ aggregation and attenuate its cytotoxicity (Guo et al. 2017b). Using thioflavin fluorescence and atomic force microscopy, it was shown that hydroxylated SWCNTs inhibited $A\beta_{42}$ fibrillogenesis (the ratio of -OH groups being critical) and disaggregated preformed amyloid fibrils and exhibited cytoprotective effects against $A\beta_{42}$ fibrillation-induced cytotoxicity (Liu et al. 2019). Dendrimer with poly(propylene imine) core and maltose-histidine shell considerably improved biocompatibility and ability to cross BBB in vivo and were found to protect synapse and memory when administered to AD transgenic mice, suggesting that they could be applied in AD prevention via synapse protection (Aso et al. 2019).

Investigation of the AuNPs coated with metal-phenolic networks on amyloid fibril showed that the coated NPs inhibited amyloid formation, the highest inhibitory activity (90%) being observed with Co-tannic acid networks, in which the geometry of the exposed cobalt coordination site facilitated its interactions with histidine and methionine residues in the Aβ peptides (Zhang et al. 2019b). Effect of Au nanospheres (AuNSs; 20 nm in diameter) and Au nanocubes (AuNCs; 20 nm in edge length) on the aggregation of $A\beta_{1\text{-}40}$ involved in AD was investigated by Wang et al. (2019). AuNSs with hybrid facets exhibited higher affinity to $A\beta_{1\text{-}40}$ due to higher degree of surface atomic unsaturation compared to the {100}-faceted AuNCs, and thus, they accelerated the fibrillation process of $A\beta_{1\text{-}40}$ more than AuNCs, which played a catalytic role in peptide nucleation through interfacial adsorption of $A\beta_{1\text{-}40}$. Therefore, AuNSs exert a more significant acceleration effect on the fibrillation process of $A\beta_{1\text{-}40}$ than AuNCs. Moreover, shape-dependent secondary structure transformation of $A\beta_{1\text{-}40}$ with different AuNPs was estimated as well. Impact of NPs on amyloid peptide and protein aggregation with a focus on AuNPs was overviewed by John et al. (2018). Gold NPs conjugated to the peptide CLPFFD could be useful for destroying toxic aggregates of Aβ (Prades et al. 2012). Green face-centered cubic crystalline, spherical- and triangular-shaped AuNPs with mean size of 30 nm fabricated using ethanolic bark extract of *Terminalia arjuna* showed dose-dependent inhibition of AChE and butyrylcholinesterase (IC_{50} values of 4.25 ± 0.02 μg/mL and 5.05 ± 0.02 μg/mL, respectively), promising antioxidative activity and also effectively suppressed the fibrillation of Aβ and destabilized the preformed mature fibrils (Suganthy et al. 2018). The human plasma biomolecular-corona-coated spherical- or rod-shaped AuNPs showed lower inhibitory effect on $A\beta_{1\text{-}42}$ fibrillation kinetics compared with CSF biomolecular-corona-coated NPs and pristine NPs. On the other hand, pristine NPs accelerated the $A\beta_{25\text{-}35}$ fibrillation process, while the corona-coated ones showed an inhibitory effect (Lotfabadi et al. 2018). Investigation of the impact of AuNPs on the conformation of $A_{16\text{-}22}$ tetramers/octamers using extensive all-atom molecular-dynamics simulations in explicit solvent showed that their addition into $A_{16\text{-}22}$ solution prevented β-sheet formation and could probably inhibit $A_{16\text{-}22}$ and full-length Aβ fibrillation (Song et al. 2018). A nanoconjugate composed of chymotrypsin, Aβ aptamer, and AuNP can actively capture Aβ through interaction between the aptamer and Aβ and destroy the target peptides through proteolysis mediated by the adjacent protease molecules, thus enabling more effec-

tive clearance of Aβ; it could be used in effective treatment of AD in the future (Li et al. 2018a).

A review paper dealing with metal-involved theranostic agents based on metal chelators, metal complexes, and metal NPs, which could be involved in fighting AD, was presented by Wang et al. (2018b). Do et al. (2016) reported an effective strategy to regulate the biodistribution of magnetic NPs in the brain through the application of an external electromagnetic field, which could be used as a targeting system for AD diagnosis and therapy. The β-CD-modified Fe_3O_4 magnetic NPs considerably inhibited fibrillization and consequent aggregation of the Aβ peptide (25–35) (Ansari et al. 2016). Dextran-coated Fe_3O_4 magnetic NPs loaded with osmotin, which were delivered to the brains of $A\beta_{1-42}$-treated mice using a functionalized magnetic field composed of positive- and negative-pulsed magnetic fields generated by electromagnetic coils, strongly mitigated $A\beta_{1-42}$-induced synaptic deficits, Aβ accumulation, β-site amyloid precursor protein cleaving enzyme 1 (BACE1) expression, and tau hyperphosphorylation, their effect being greater than that of free osmotin (Ul Amin et al. 2017). Glycine-coated superparamagnetic NPs (glycine: Fe_3O_4 = 5:1) destroyed lysozyme, α-lactalbumin, and insulin amyloid fibrils in concentration-dependent manner, but not the bovine α-crystallin amyloid fibrils (Antosova et al. 2019). After addition of magnetic Fe_3O_4 NPs showing sizes 5 and 20 nm, respectively, to the solution of lysozyme amyloid fibrils, the interaction started immediately and their adsorption and agglomeration onto the fibrillary surfaces was observed. The Fe_3O_4 NPs with a diameter size of 10 nm enabled segmentation of the slender fibrils into debris, suggesting complete destruction of the amyloid fibrillary structure (Tomasovicova et al. 2018). Gumpelmayer et al. (2018) reported that magnetite Fe_3O_4 has no intrinsic peroxidase activity, and therefore, it is probably not involved in oxidative stress causing damage of brain neurons of patients with AD. As a promising brain drug delivery system in treatment of AD, the magnetic Fe_3O_4 NP-loaded PEG-PLGA nanocomposite modified with antitransferrin monoclonal antibody (OX26) receptor was described (Cui et al. 2018). After IV injection, the magnetic NPs functionalized with an antiferritin antibody NPs were able to recognize and bind specifically to the ferritin protein accumulated in the subiculum in the hippocampal area of the AD transgenic mice and could be used for detection of ferritin accumulation in AD (Fernandez et al. 2018).

Gao et al. (2016) prepared a polyoxometalate-based nanozyme displaying both protease-like activity for depleting Aβ aggregates and SOD-like activity for scavenging Aβ-mediated ROS, which was able to remove Cu from Cu-induced Aβ oligomers by chelatation. Sulfur NPs showing volute-like, tadpole-like, and sphere-like morphologies coupled with brain-targeting peptides RVG reduced the $A\beta\text{-}Cu^{2+}$ complex aggregation, increased SH-SYSY cell viability, and caused improvements in learning disability and cognitive loss in the transgenic AD mice model, with spherical NPs being the most effective (Sun et al. 2018). $A\beta_{1-40}$ was arrested at the oligomeric stage in the presence of zeolite containing Zn^{2+} or Cu^{2+}, while it continued to the fibrillary state in the presence of zeolites containing other cations, suggesting that zeolites can impact the aggregation and speciation of amyloids (Lucas

and Keitz 2018). The iminodiacetic acid-modified human lysozyme (hLys) showing strong binding affinity for Zn^{2+} considerably mitigated Zn^{2+}-mediated Aβ aggregation and cytotoxicity, and they were found to protect Aβ from Zn^{2+}-induced aggregation and rapidly depolymerize Zn^{2+}-Aβ aggregates (Li et al. 2018b). Zn-loaded NPs conjugated with a glycopeptide consisting of 7 amino acids (g7), able to deliver Zn into the brain across the BBB, which were injected in APP23 mice, showed notable reduction in plaque size, affected the proinflammatory cytokines IL-6 and IL-18, and two weeks after NPs administration normalized the observed hyperlocomotion of APP23 mice (Vilella et al. 2018). Zhan et al. (2019) designed thymine-modified Zn phthalocyanine (T-ZnPc) that could specifically recognize Fe^{3+} and Al^{3+} ions and show strong affinity to these ions found at pronouncedly high levels on and around Aβ protofibrils, compared with the unaffected part of the brain. The T-ZnPc, when used for photodynamic therapy, generates abundant ROS, resulting in significant degradation of Aβ protofibrils. Moreover, the chelation effect could reduce the free Fe^{3+} and Al^{3+} concentration in the brain and inhibit the formation of new Aβ protofibrils.

Yang et al. (2018c) combined chlorogenic acid (CGA) with SeNPs and the formed CGA@SeNPs showed enhanced inhibitory effect on $Aβ_{40}$ aggregation and protected PC12 cells from Aβ aggregation-induced cell death and it could be assumed higher efficiency of CGA@SeNPs in reducing $Aβ_{40}$ toxicity in long-term use compared to free antioxidant CGA. In highly biocompatible and hemocompatible penetratin peptide-loaded PEG-stabilized gold nanostars modified with ruthenium complex, the Ru(II) complexes as luminescent probes for tracking drug delivery inhibited the formation of Aβ fibrils and dissociated preformed fibrous Aβ under the near-infrared (NIR) irradiation. It was found that the penetrating peptide could enhance the delivery of the NPs to the brain in vitro and in vivo (Yin et al. 2016). Small-sized Pd hydride NPs were applied to secure in situ sustained hydrogen release in the AD brain, whereby this bioreductive hydrogen was able to recover mitochondrial dysfunction and inhibit Aβ generation and aggregation. These NPs could reduce oxidative stress by scavenging harmful •OH radicals in a self-catalysis way, which contributed to the amelioration of the cognitive impairment in AD mice (Zhang et al. 2019c).

Multifunctional MoO_{3-x} nanodots showed superb catalase and SOD mimic activities due to efficient charge transition between Mo^{5+}/Mo^{6+} on their surface, and inhibited Aβ aggregation and destabilized the preformed fibrils. They also protected neuronal cells from apoptosis induced by Aβ (Han et al. 2019). The monomeric and dimeric forms of the small amyloid peptides adopt a random coil conformation in the presence of MoS_2, while the helical form is preferable for the monomeric form and the β-sheet and helix forms are the preferable forms for dimers in aqueous solution. The confinement of MoS_2 promotes deaggregation of Aβ peptides rather than aggregation (Mudedla et al. 2018). Classical molecular dynamics simulations used to study of the binding modes and structural properties of amyloid fibrils at the interface of molybdenum disulphide (MoS_2) nanotubes and the nanosurface showed that MoS_2 nanotubes induced disruptions in the structure of the amyloid fibrils reflected in changes of the β-sheet conformation of the fibrils to a turned conforma-

tion, the effect being stronger in the case of nanotubes. The destabilization of the fibrils was connected with reduced intermolecular H-bonds as well as hydrophilic and hydrophobic contacts between the monomer peptides in the fibril due to their adsorption onto the MoS_2 materials. Thus, the MoS_2-based materials could be used not only to inhibit the aggregation of smaller protofibrils to matured fibrils but also to bust the already formed fibrils (Mudedla et al. 2019).

The functionalization of gadolinium-based magnetic resonance imaging NPs with peptides highly specific for Aβ fibrils, specifically LPFFD (which binds to the central hydrophobic region of Aβ) and KLVFF (which is essential for the formation of β-sheet structures and binds to the full-length Aβ peptide via an atypical antiparallel β-sheet motif), was reported as a useful multimodal imaging tool to selectively discriminate and diagnose amyloidoses (Plissonneau et al. 2016). Sadowska-Bartosz and Bartosz (2018) discussed potential applications of redox NPs (RNPs), such as CeO_2 RNPs, boron cluster-containing and SiO_2-containing RNPs, Gd_3N@C80 encapsulated RNPs, and concentrates on nitroxide-containing RNPs, for treatment of neurodegenerative diseases; as most promising was found application of RNPs containing covalently bound nitroxides characterized with and good penetration ability through the BBB.

Ultrasound-excited protoporphyrin IX-modified oxidized mesoporous carbon nanospheres containing PEG and RVG prepared as multifunctional NPs showing strong inhibitory effect of tau protein phosphorylation and Aβ aggregation pronouncedly enhanced the cognitive level of APP/PS1 transgenic mice, achieving dual-target inhibition of AD (Xu et al. 2018). Porphyrinic metal-organic framework PCN-224 NPs synthesized by coordinating tetrakis(4-carboxyphenyl)porphyrin ligands with zirconium and showing high photo-oxygenation efficiency after activation by NIR light successfully inhibited self-assembly of monomeric Aβ into a β-sheet-rich structure and notably reduced Aβ-induced cytotoxicity. In primary cultured hippocampal neurons, SiNPs accumulated in endolysosomes and caused a rapid and persistent deacidification of endolysosomes, notably reduced endolysosome Ca stores, and increased $A\beta_{1\text{-}40}$ secretion, which could be linked to increased amyloidogenesis, and therefore, SiNPs might not be considered to be appropriate nanomaterial for therapeutic strategies against AD and other neurological disorders linked to endolysosome dysfunction (Ye et al. 2019).

Because phospholipids play an important role in memory and learning abilities and act as a source of choline in ACh synthesis, CS/phospholipid/β-CD microspheres were prepared and administered to rats, and it was found that they significantly improved the learning and memory abilities of the animals, attenuated the expression of protein kinase C-δ, and inhibited the activation of microglias, suggesting their potential in the treatment of AD (Shan et al. 2016). Nanoliposomes containing phosphatidylcholine, cholesterol, and phosphatidic acid prevented $A\beta_{42}$ amyloid formation, reversed $A\beta_{42}$-induced human microvascular endothelial dysfunction, and could be useful in AD therapy (Truran et al. 2016).

Fermented soybean nanonutraceuticals increased the activity of AChE, while reducing the activity of GSH, SOD and catalase, decreased the level of lipid peroxidation and protein carbonyl contents and reversed colchicine-induced behavioral

and neurochemical alterations in rats through strong antioxidant activity. The results of in silico studies showed that these nanonutraceuticals could also pronouncedly inhibit Aβ and BACE1, and thus, they might have beneficial impact on cognitive defects associated with AD (Bhatt et al. 2018). Reducing expression of BACE1, a key enzyme cleaving the amyloid precursor protein to develop AD, in central neuron through RNA interference technology could be utilized to overcome AD. The siRNA nanocarriers based on PEGylated poly(2-(*N,N*-dimethylamino)ethylmethacrylate) modified with both the CGN peptide and Tet1 peptide to improve BBB and neuron-specific binding were designed by Wang et al. (2018c). These control target siRNA complexes pronouncedly reduced BACE1 mRNA and the amyloid plaques, suppressed phosphorylated tau protein levels and stimulated hippocampal neurogenesis, and were able to restore the cognitive performance of the AD in transgenic mice to the level of wild-type control, avoiding negative effects on myelination. Investigation of the neuroprotective effect of nanodiamond (ND; adamantine-based NPs) in Al-induced cognitive impairment in rats used as an experimental model of AD showed that ND attenuated the increase of hippocampal $A\beta_{42}$ and BACE1, down-regulated the phosphorylation of tau protein, and improved learning and memory and reversed histological alterations. The protective effect of ND against memory deficits and AD-like pathological aberrations was assumed to be accomplished via modulating nuclear factor-κB (NF-κB) and signal transducer and activator of transcription 3 (STAT3) signaling (Alawdi et al. 2017). A novel redox-activated NIR responsive nanoplatform based on polyoxometalates (POMs) capable of generating local hyperthermia due to disaggregation of Aβ fibrils under NIR laser irradiation because of strong NIR absorption by POMs was developed by Ma et al. (2018). The antioxidant activity of this nanoformulation was reflected in Aβ-induced ROS scavenging. Tetrahedral DNA nanostructures were found to protect and rescue PC12 cell death through $A\beta_{25\text{-}35}$-induced PC12 cell apoptosis, affected the abnormal cell cycle, restored normal nuclear morphology and caspase activity, resulting in notably improved apoptosis, and were able to prevent the damage caused by Aβ deposition by activating the ERK1/2 pathway (Shao et al. 2018).

Biocompatible nanogels composed of a polysaccharide pullulan skeleton with hydrophobic cholesterol moieties (cholesterol-bearing pullulan) as artificial chaperones were found to incorporate up to 6–8 $A\beta_{1\text{-}42}$ molecules per particle and induce a change in the Aβ conformation from a random coil to α-helix- or β-sheet-rich structure. The modification of the nanogels with an amino-group led to more effective inhibition of the aggregation of $A\beta_{1\text{-}42}$ due to electrostatic interactions. Besides, the nanogels protected PC12 cells from Aβ toxicity (Ikeda et al. 2006). Loureiro et al. (2016) developed PLGA NPs surface functionalized with antitransferrin receptor monoclonal antibody (OX26) and anti-Aβ (DE2B4) to deliver the encapsulated peptide iAβ5 into the brain. Using porcine brain capillary endothelial cells as a BBB model, it was found that the uptake of these immune NPs demonstrating controlled delivery of the peptide iAβ5 was substantially elevated compared to the NPs without monoclonal antibody functionalization.

A self-destructive nanosweeper based on multifunctional peptide polymers that is capable of capturing and clearing Aβ, which recognizes and binds Aβ via co-

assembly through H-bonding interactions, was described by Luo et al. (2018). The Aβ-loaded nanosweeper could enter cells and upregulate autophagy resulting in stimulation of the Aβ degradation and rescue memory deficits of AD transgenic mice. Thus, it could be utilized in the treatment of AD. Treatment of AD-like transgenic mice with PEGylated NPs surface-functionalized with an antibody directed against $A\beta_{1\text{-}42}$ resulted in complete correction of the memory defect, considerable reduction of the Aβ soluble peptide and its oligomer level in the brain, and pronounced increase of Aβ levels in plasma (Carradori et al. 2018).

Pulmonary administration of liposomes functionalized with phosphatidic acid and an ApoE-derived peptide considerably reduced Aβ in the brain of AD mice, indicating that lung administration could be used as an alternative for noninvasive brain delivery of NPs designed for AD therapy (Sancini et al. 2016). CS-coated PLGA NPs conjugated with a novel anti-Aβ antibody demonstrated improved uptake at the BBB and better targeting of the Aβ proteins deposited in the cerebral amyloid angiopathy (CAA) model in vitro as compared with the control nanovehicles, and could be used as immuno-nanovehicles to image CAA deposits in vivo (Jaruszewski et al. 2012). Zhang et al. (2014a) designed a dual-functional NP drug delivery system based on a PEGylated PLA polymer, to the surface of which two targeting peptides, TGN (specifically targeting ligands at the BBB) and QSH (showing good affinity with $A\beta_{1\text{-}42}$, the main component of amyloid plaque), were conjugated, which might be a valuable targeting system for AD diagnosis and therapy. To modulate the neuroinflammation for preventing or delaying the neurodegenerative process triggered by Aβ, Frozza et al. (2013b) used a combination of RES and a lipid-core nanocapsule-based delivery system. Intranasally administered liposomes loaded with H102, a novel β-sheet breaker peptide, exhibited 2.92-fold larger AUC in the hippocampus than the solution group, excellently ameliorated spatial memory impairment in AD model rats, and inhibited plaque deposition, even at a lower dosage compared with H102 IN solution (Zheng et al. 2015). Lf-bearing generation 3 polypropylenimine dendrimer led to 2.1-fold higher DNA uptake than the unmodified dendriplex in bEnd.3 murine brain capillary endothelial cells in vitro and, following IV administration in vivo, significantly increased gene expression in the brain (>6.4-fold higher compared to that of the unmodified dendriplex) was observed, while in the lung and the kidneys, the gene expression decreased (Somani et al. 2015). Jaruszewski et al. (2014) prepared nanovehicles with the antiamyloid antibody (IgG4.1) grafted on the surface capable to target cerebrovascular amyloid and serve as an early diagnostic and therapeutic agent. The nanovehicles were loaded with resonance imaging contrast agents and carried either anti-inflammatory and antiamyloidogenic agents (e.g., CUR) or immunosuppressants (e.g., dexamethasone), and demonstrated very good distribution within the brain vasculature. Basic fibroblast growth factor loaded PEG-PLGA NPs modified with *Solanum tuberosum* lecitin that selectively binds to *N*-acetylglucosamine on the nasal epithelial membrane significantly improved the spatial learning and memory of AD affected rats compared to the control model group. Their IN administration could be used for brain delivery of basic fibroblast growth factor to treat AD (Zhang et al. 2014b). B6 peptide-modified PEG-PLA NPs (B6 NPs) showed significantly higher accumula-

tion in brain capillary endothelial cells through lipid raft-mediated and clathrin-mediated endocytosis than PEG-PLA NPs, and the administration of the neuroprotective peptide NAPVSIPQ encapsulated in B6 NPs in an AD mouse model led to excellent amelioration of learning impairment, cholinergic disruption, and loss of hippocampal neurons even at a lower dose (Liu et al. 2013a). Sialic acid-modified SeNPs conjugated with the B6 peptide having high permeability across the BBB were highly taken up by cerebral endothelial cells (bEnd3), and although they could not sufficiently inhibit Aβ aggregation, they could disaggregate pre-formed Aβ fibrils into nontoxic amorphous oligomers, indicating that these NPs could be applied in the treatment of brain diseases (Yin et al. 2015). Also Lf-modified PEG-co-polycaprolactone NPs were proven to be suitable for the improved brain delivery of the neuroprotective NAPVSIPQ peptide using IN administration, and memory improvement was observed even at the lower dose (Liu et al. 2013b). Following IN administration, a nanostructure of monosialotetrahexosylganglioside-modified reconstituted high-density lipoprotein (GM1-rHDL), having antibody-like high-binding affinity to Aβ, loaded with the neuroprotective peptide (NAP) protected neurons from $A\beta_{1\text{-}42}$ oligomer/glutamic acid-induced cell toxicity in vitro, reduced Aβ deposition, ameliorated neurological changes, and rescued memory loss more efficiently than αNAP solution and GM1-rHDL in AD model mice (Huang et al. 2015). The ability of liposomes bi-functionalized with phosphatidic acid and an ApoE-derived peptide to withdraw amyloid peptides from the brain was reported by Mancini et al. (2016).

About four- to five-fold increase in the inhibition efficiency of human lysozyme was observed by the amino modification, which could be connected with more widely distributed positive charges of basified human lysozyme (hLys-B), which promoted broad electrostatic interactions between Aβ and hLys-B. At low concentrations of hLys-B with a modification degree of 4.4, the conformational transition of Aβ to β-sheet structures was suppressed, resulting in changes in the aggregation pathway and the formation of Aβ species showing less cytotoxicity (Li et al. 2018c). Conjugation of hydrophobic heptapeptide Ac-LVFFARK-NH2 to β-CD suppressed the conformational transition of Aβ and showed stronger inhibitory effect on Aβ fibrillation than heptapeptide alone, suggesting that this conjugate exhibited protective effect on $A\beta_{40}$-induced cytotoxicity (Zhang et al. 2018b).

4.4.3 Effect of NPs on Tau Protein

Tau (tubulin-associated unit) protein is a designation for a group of related proteins of varying length, with six main isoforms being produced in the CNS via alternative splicing. The most salient function of tau proteins is to bind to microtubules, thus stabilizing them and making them more readily polymerizable; the protein possesses numerous other functions, with more being discovered on an ongoing basis (Sotiropoulos et al. 2017). Tau proteins in AD are deposited in the brain, where they are in hyperphosphorylated form, and as a result, tau molecules cluster into helical

fibers. In the so-called amyloid plaques, the Aβ core is typically wrapped with neurofibrillary fibers from tau proteins. Then, together, Aβ and tau represent two basic biochemical symptoms of AD in the brain (Iqbal et al. 2010; Guo et al. 2017c), though neurofibrillary pathology is the component that is most closely associated with disease severity and progression (Nelson et al. 2012; Murray et al. 2015). In addition, Chiu et al. (2014) found that plasma tau levels were negatively associated with the performance of logical memory, visual reproduction, and verbal fluency, volume of total gray matter, hippocampus, and amygdala, and gray matter densities of various regions. The function of pathologically affected tau protein is illustrated in Fig. 4.3.

Phosphatidylserine-functionalized SLNPs loaded with histone deacetylase inhibitor nicotinamide with optimized particle size of 124 ± 0.8 nm showed improved brain delivery and when administered via i.p. injection were found to improve the cognition, preserve the neuronal cells, and reduce tau hyperphosphorylation in a rat model of AD. This formulation was more effective than the conventional oral administration of nicotinamide at the early stage of AD (Vakilinezhad et al. 2018).

CUR-encapsulated PLGA NPs at a ratio 50:50 were reported to prevent the phosphorylation of Akt and tau proteins in SK-N-SH cells induced by H_2O_2 and showed improved anti-inflammatory and antioxidant activities compared to free CUR (Paka et al. 2016). CUR-loaded brain-targeting PLGA-PEG NPs conjugated with B6 pep-

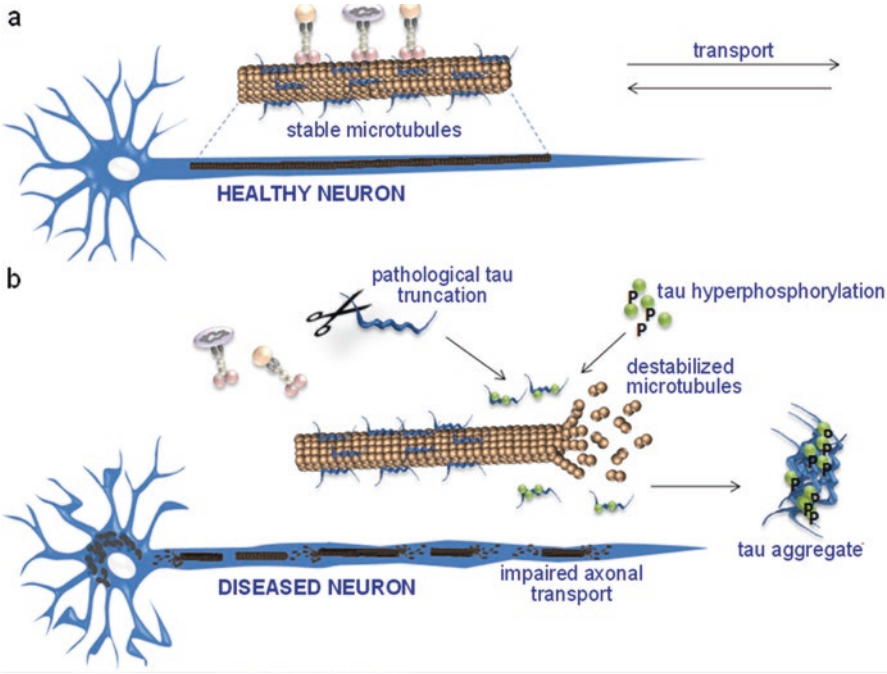

Fig. 4.3 Healthy and affected neuron and pathogenic function of tau protein

tide strongly improved the spatial learning and memory capability of APP/PS1 transgenic mice compared with native CUR, and in the ex vivo assays, it was found that this formulation reduced hippocampal Aβ formation and deposition and tau hyperphosphorylation, suggesting its potential to be used for the treatment of AD (Fan et al. 2018b). Quercetin-encapsulated liposomes grafted with RMP-7, a bradykinin analog, and Lf caused considerable reduction of Aβ-induced neurotoxicity, improved the viability of SK-N-MC cells and significantly inhibited the expression of phosphorylated c-Jun *N*-terminal kinase, phosphorylated p38, and phosphorylated tau protein at Ser_{202} by SK-N-MC cells, suggesting that such liposomes are a promising carrier targeting the BBB to prevent Aβ-insulted neurodegeneration (Kuo and Tsao 2017). Cardiolipin (CL)-conjugated liposomes carrying CUR inhibited the expression of phosphorylated p38, phosphorylated c-Jun N-terminal kinase, and phosphorylated tau protein at Ser_{202} and prevented neurodegeneration of SK-N-MC cells. Wheat germ agglutinin-grafted-CL-conjugated liposomes carrying CUR or CUR and nerve growth factor (NGF) notably improved the permeation of CUR and NGF across the BBB, reduced Aβ plaque deposition and the malondialdehyde level, and increased the percentage of normal neurons and cholinergic activity in the hippocampus of AD rats (Kuo et al. 2017). Polyacrylamide-cardiolipin-PLGA NPs grafted with surface 83–14 monoclonal antibody co-loaded with rosmarinic acid and CUR showing good BBB permeability were able to enhance the viability of SK-N-MC cells insulted with Aβ deposits achieving the levels of phosphorylated p38 and phosphorylated tau protein at Ser_{202} similar to control, suggesting that such NPs had promising potential to be used in pharmacotherapy to permeate the BBB and reduce the fibrillar tau-induced neurotoxicity (Kuo and Tsai 2018).

Both SWCNTs and multiwalled carbon nanotubes (MWCNTs) with different surface tension impaired the viability and complexity of PC12 cells by induction of necrotic modes of cell death (MWCNTs) or activation apoptotic modes of cell death (SWCNTs); however, more structural changes of tau protein were observed at application of SWCNTs (Zeinabad et al. 2016). Formulation of an encapsulated small peptide derived from BMP-9 (SpBMP-9) that can stimulate differentiation of cholinergic neurons and inactivate glycogen synthase kinase 3β and tau kinase in alginate/CS NPs with mean diameter approx. 240 nm increased the viability of SH-SY5Y cells compared to the control. SpBMP-9 released from these NPs stimulated the SH-SY5Y differentiation into mature neurons, as demonstrated by a higher neurite outgrowth and an increased expression of the neuronal markers neuron-specific enolase and vesicular ACh transporter (Lauzon et al. 2018).

Investigation of the interaction of folic acid functionalized AuNPs and Au-shelled Fe_3O_4 NPs with microtubule and microtubule-associated protein tau showed that hydrophobic interaction is a driving force for AuNPs binding to tubulin and tau, whereby the affinity of these AuNPs to both tau and tubulin increased with increasing temperature; the investigated AuNPs were able to stabilize microtubule polymers. Hence, the microtubule network could be regarded as a potential candidate for targeting by AuNP-assisted photothermal therapy (Ghalandari et al. 2019). Treatment of anthocyanin-loaded PEG-AuNPs ameliorated memory impairments in the $A\beta_{1\text{-}42}$ injected mice to greater extent than treatment with free anthocyanins, and

this nanoformulation protected pre- and postsynaptic proteins from $A\beta_{1-42}$-induced synaptic dysfunction, prevented the hyperphosphorylation of tau protein at Ser_{413} and Ser_{404}, and inhibited apoptosis and neurodegeneration in the $A\beta_{1-42}$-injected mice (Ali et al. 2017). AgNPs with particle diameter approximately 10–20 nm form a static complex with tau protein via hydrogen bonds and van der Waals interactions, inducing slight changes on the tau protein structure. Their adverse effect on SH-SY5Y neuroblastoma cell was reflected in cell mortality through fragmentation of DNA resulting in apoptosis (Rahmani et al. 2018). Citrate-coated AgNPs (20 nm) exhibited neurotoxicity in glutamatergic neurons derived from human embryonic stem cells, which was reflected in strong damage of neurite outgrowths, increased ROS production, Ca^{2+} influxes, activation of calmodulin, the induction of nitric oxide synthase, and increased phosphorylation of glycogen synthase kinase-3α/β at Tyr_{216} and tau at Ser_{396}, while reducing the expression of tau46 (Begum et al. 2016). Metals, such as Cu, Fe, Zn, Mg, and Al, were reported to be involved in the pathogenesis of AD. These metal ions could pronouncedly affect the processes of Aβ deposition in senile plaques and the inclusion of phosphorylated tau in neurofibrillary tangles. Their adverse impact is connected with oxidative stress, neurotoxicity, autophagy, and apoptosis and results in cognitive decline. Therefore, developing effective chelators specific for metal ions is very important (Wang and Wang 2017). In vitro study of interaction of FeNPs with tau protein showed that there is almost one binding site of FeNP per protein. In this spontaneous interaction, hydrogen bonds and van der Waals interactions play a dominant role. Via these, the FeNPs also stabilized the random coil structure of tau protein. Moreover, FeNPs were found to reduce PC12 cell viability by fragmentation of DNA in an apoptotic manner (Hajsalimi et al. 2018). Al_2O_3 NPs were found to bind to the hydrophilic residues of tau protein by forming a static complex and also fold the structure of tau toward a more packed structure. Al_2O_3 NPs also induced cytotoxicity against SH-SY5Y cells, and they could elicit cell mortality through membrane leakage, caspase-9/-3 activations, and induction of both apoptosis and necrosis (Kermani et al. 2018). NiO NPs increased the hydrophobic portions of tau protein, changed its secondary and tertiary structure, induced the formation of amorphous tau aggregates, and exhibited strong impact on generating intracellular ROS and apoptosis induction (Hosseinali et al. 2019). Biothermodynamic and molecular studies focused on the effects of NiO NPs on tau protein and neuron-like cells confirmed that NiO NPs may mediate the formation of electrostatic interactions with tau and induction of undesired harmful effects on neurons (Hajimohammadjafartehrani et al. 2019). Mn_2O_3 NPs were found to fold the structure of tau toward a more packed structure and were cytotoxic against SH-SY5Y cells, which was reflected in decreased cell viability, caspase-3 and caspase-9 activation, Bax/Bcl-2 ratio elevation, and apoptosis induction (Mehdizadeh et al. 2019). A formation of a static complex of cobalt oxide NPs (Co_3O_4 NPs) with tau protein through hydrogen bonds and van der Waals forces was reported by Nouri et al. (2018), whereby important roles of Ser and Gln residues in the formation of hydrogen bonds between tau protein and Co_3O_4 NPs was confirmed by a docking study. Co_3O_4 NPs induced alterations on the tertiary and secondary structure of tau protein and were able to change the unfolded struc-

ture of tau protein toward a more folded conformation. Moreover, Co_3O_4 NPs reduced PC12 cell viability through membrane leakage, fragmentation of DNA, apoptosis, and necrosis.

The treatment of SiNPs impaired the morphology of human SK-N-SH and mouse neuro2a neuroblastoma cells, decreased cell density and cell viability, induced cellular apoptosis, and elevated the levels of intracellular ROS. SiNPs also increased the deposit of intracellular $A\beta_{1\text{-}42}$ and enhanced phosphorylation of tau at Ser_{262} and Ser_{396} but up-regulated the expression of amyloid precursor protein and down-regulated the Aβ degrading enzyme neprilysin in treated cells (Yang et al. 2014b). SiO_2 NPs were found to induce conformational changes of tau protein resulting in partially folded and amorphous aggregated structure and had negative effect on SH-SY5Y cell integrity, causing oxidative stress and apoptosis in this neuroblastoma cell line (Roshanfekrnahzomi et al. 2019). Tau protein was found to reduce the colloidal stability of SiO_2 NPs that can bind to the tau protein with high affinity through hydrogen bonds and van der Waals interactions. A docking study also showed that Ser, Thr, and Tyr residues provide a polar microenvironment for SiO_2 NPs/tau interaction. Moreover, SiO_2 NPs induced mortality of PC12 cells through both apoptosis and necrosis mechanisms (Shariati et al. 2018). Intranasal instillation of fluorescein isothiocyanate-tagged SiO_2 NPs to 3-month-old male C57BL/6 N mice resulted in decreased social activity after 1 and 2 months of exposure as well as anxiety and cognitive impairment after 2-month exposure. They increased tau phosphorylation and neuroinflammation, altered the expression of important synapse structural proteins, and were observed in the brains of SiO_2 NPs-exposed mice as well (You et al. 2018b). Treatment of human neuroblastoma cell line (SH-SY5Y) with TiO_2 NPs resulted in lower growth rate and a higher shortening rate of microtubules, as well as shortened lifetimes of de novo microtubules. Although TiO_2 NPs did not affect the expression and phosphorylation state of tau proteins, they interacted with tubule heterodimers, microtubules, and tau proteins, resulting in the instability of microtubules, which contributed to the neurotoxicity of TiO_2 NPs (Mao et al. 2015). TiO_2 NPs were found to cause secondary and tertiary structural changes, and the formation of amorphous tau aggregates, whereby the denatured adsorbed protein on the NP surface may enhance TiO_2 NPs cytotoxicity (Fardanesh et al. 2019).

4.5 Other Diseases

4.5.1 Huntington's Disease

Huntington's disease (HD) is a genetic disorder caused by a CAG expansion mutation in the Huntingtin gene leading to polyglutamine (polyQ) expansion in the N-terminal part of the huntingtin (Httex1) protein. Polyglutamine expansion within the N-terminal region of the huntingtin protein results in the formation of intracel-

lular aggregates responsible for HD. Ceccon et al. (2019) found that TiO_2 NPs decreased aggregation of huntingtin peptides comprising the N-terminal amphiphilic domain with a ten residue C-terminal polyglutamine tract by catalyzing the oxidation of methionine to a sulfoxide, leading to an aggregation-incompetent peptide, which suggests that the catalysis of methionine oxidation within the N-terminal domain of the huntingtin protein may potentially provide a strategy for delaying the onset of HD.

PLGA NPs coated with polysorbate 80, which were loaded with polyglutamine aggregation inhibitors, peptides QBP1, NT17, and PGQ9P2, showed dose-dependent inhibition of polyglutamine aggregation in Neuro 2A and PC12 cells and improved motor performance in *Drosophila* model of HD (Joshi et al. 2019). Treatment with thymoquinone-loaded SLNPs (TQ-SLNs) pronouncedly improved function of ATPases in 3-nitropropionic acid (3-NP) intoxicated rats at doses 10 and 20 mg/kg, respectively; mitigated the overexpression of glial fibrillary acidic protein, pro-inflammatory cytokines, and p-p65 NF-κB nuclear translocation; improved tyrosine hydroxylase immune reactive neurons; and ameliorated the motor abnormalities and neuroinflammation in rat model of Huntington's disease, being efficacious at lower doses compared with thymoquinone suspension (Ramachandran and Thangarajan 2018). The TQ-SLNs pronouncedly alleviated the levels of oxidative stress, restored the antioxidant defense system, controlled the mitochondrial succinate dehydrogenase inhibition, and mitigated the anticholinergic effect induced by 3-NP induction (Ramachandran and Thangarajan 2016). The calmidazolium chloride (CLC) complex with bovine serum albumin (BSA) can improve Httexl aggregation inhibition by interacting with the aggregation initiator, and the NT17 part of Httex1 and CLC-loaded BSA NPs reduced the polyglutamine aggregates in HD-150Q cells (Singh et al. 2018c).

The NPs with a 6-nm iron oxide core and a zwitterionic polymer shell containing similar to 5–12 wt% covalently linked trehalose with particle sizes 20–30 nm were by 3–4 orders more efficient in inhibiting protein fibrillation in extra-cellular space, in blocking aggregation of polyglutamine-containing mutant huntingtin protein in model neuronal cells, and in suppressing mutant huntingtin aggregates in HD mouse brain than molecular trehalose. At 80–200 trehalose molecules per NP, efficient brain targeting, entry into neuronal cells, and suppression of mutant huntingtin aggregation could be observed (Debnath et al. 2017).

An overview of stem cell therapy for the neurodegenerative disorder in HD with focus on novel nano- and microcarriers capable of delivering proteins, nucleic acids, and cells was presented by Andre et al. (2016).

Repeated systemic delivery of biocompatible polymeric NPs modified with glycopeptides (g7) and loaded with cholesterol (g7-NPs-Chol) efficiently crossed the BBB and localized in glial and neuronal cells in different brain regions, rescued synaptic and cognitive dysfunction, and partially improved global activity in HD mice (Valenza et al. 2015). Optimized rosmarinic acid-loaded SLNPs (average size of 149.2 ± 3.2 nm, zeta potential of −38.27 mV) notably improved behavioral abnormalities and attenuated the oxidative stress in 3-NP-treated rats, nasal delivery showing considerably higher therapeutic action than IV administration (Bhatt et al.

2015). CUR encapsulated SLNPs pronouncedly increased the activity of mitochondrial complexes and cytochrome levels, restored the GSH levels and SOD activity, notably reduced mitochondrial swelling, lipid peroxidation, protein carbonyls, and ROS in 3-NP-treated rats, and caused also considerable improvement in their neuromotor coordination (Sandhir et al. 2014). Godinho et al. (2013) investigated self-assembling of modified β-CD NPs as neuronal siRNA delivery vectors with a focus on HD and found that these complexes reduced the expression of the huntingtin protein gene in rat striatal cells and in human HD primary fibroblasts, and repeated brain injections of CD-siRNA complexes resulted in selective attenuation of motor deficits in R6/2 mouse model of HD.

4.5.2 Wilson's Disease

Wilson's disease is characterized by excessive Cu accumulation in the liver and the brain, resulting in liver disease and neuropsychiatric symptoms. Encapsulation of a copper chelating agent, triethylenetetramine (TETA) into surface-modified liposomes with particle sizes in the range from 139.4 ± 1.9 nm to 171.1 ± 3.5 nm resulted in 16-fold higher brain uptake of Cu chelator in rats in vivo and TETA concentrations being high enough to treat Wilson's disease (Tremmel et al. 2016). Ion-exchange reaction between $K_2Zn_3[Fe(CN)_6]_2$ NPs and Cu^{2+} ions results in high selectivity of such NPs for copper in the presence of the other endogenous metal ions. Because $K_2Zn_3[Fe(CN)_6]_2$ NPs could be readily internalized by cells to target intracellular Cu^{2+} ions for selective copper detoxification, they have potential to be applied in treatment o WD (Kandanapitiye et al. 2015a). The PEGylated Au@$ZnMoS_4$ core shell NPs which could penetrate the cell membrane to selectively remove Cu^{2+} ions from HepG2 cells in the presence of other endogenous and biologically essential metal ions could be used as cellular copper detoxifying drug for WD (Perera et al. 2013). For selective Cu detoxification in the presence of the other divalent essential metal ions including Zn(II), Fe(II), Mn(II), Ca(II), and Mg(II), biocompatible D-penicillamine conjugated AuNPs, which can readily penetrate the cell membrane to target intracellular free Cu^{2+} ions, could be used in treatment of diseases and disorders characterized with Cu overload (Kandanapitiye et al. 2015b).

4.5.3 Amyotrophic Lateral Sclerosis

ALS is a fatal neurodegenerative disease affecting the upper and lower motor neurons in the motor cortex and spinal cord. Abnormal accumulation of mutant SOD-1 in motor neurons is a pathological hallmark of some forms of the disease (Chen et al. 2017; Siddiqi et al. 2018). There is no ALS treatment, only palliative care is available to patients with ALS (Choi and Lee 2013), nevertheless riluzole was approved by the FDA for application during ALS. This derivative of benzothia-

zole may increase survival by 2–3 months by delaying the onset of ventilator dependence or tracheostomy by means of blocking tetrodotoxin-sensitive sodium channels, the kainate and NMDA receptors (Drug Bank 2019). Increased effect of riluzole by means of enhanced bioavailability using peptide nanocarriers is discussed in a review by Mazibuko et al. (2015). Carbon NPs with targeted distribution of riluzole was described by Bondi et al. (2010). Also minocycline, inhibitor of caspase-1 and caspase-3, can be administered during ALS (Zhu et al. 2002). Nanoliposome loaded minocycline and modified by lipopolysaccharide was designed by Nicholas et al. (2012). This nanosystem showed to be suitable for targeting to toll-like receptor 4 on the microglia cell in SOD-1–G93A transgenic mice. Human serum albumin NPs suitable for encapsulation of drugs is another example for nanocarriers (Mo et al. 2007). Carboxyfullerene nanotube-loaded SOD also demonstrated neuroprotective properties (Ali et al. 2008).

4.5.4 Multiple Sclerosis

MS is a chronic autoimmune disease of unknown etiology, in which the human immune system attacks the CNS and causes demyelination. Immunomodulatories or immunosuppressants, for example, corticosteroids, interferon beta, glatiramer acetate, mitoxantrone, natalizumab, fingolimod, teriflunomide, alemtuzumab, azathioprine, and cyclophosphamide, have been approved as disease-modifying drugs (Gajofatto and Benedetti 2015; Ojha and Kumar 2018).

Pomegranate seed oil administered in an animal study as a nanodroplet formulation showed an encouraging outcome in the treatment of MS (Jin et al. 2011). A water-soluble fullerene loaded an *N*-methyl-D-aspartate receptor antagonist inhibited MS disease progression (Basso et al. 2008). Carbon nanowires and nanotubes are also tested for neural repairing and regeneration process. Carbon nanotubes due to their electrical, mechanical, and chemical properties can be applied in nerve tissue engineering for treatment of MS for nongenetic manipulations of neuronal performance and network signaling in vivo (Liu et al. 2011b; Fazil et al. 2012b; Nunes et al. 2012). Emerging concept of neuroprotection through nanotechnology was reviewed by Singh et al. (2012) and Ojha and Kumar (2018).

4.6 Nanobiosensors for Detection Neurodegenerative Diseases

Sensitive biosensors are used in addition to blood tests to diagnose the degree/severity of the disease and monitor the effectiveness of the treatment. The following section provides a brief overview of the application and screening options. Review papers focused on the recent progress of aptamer systems' applications in AD bio-

markers in biosensing and novel strategies used for signal amplification in sensing AD biomarkers were presented by Shui et al. (2018a, b). The potential of dendrimers of nanoscale dimensions showing nanoscopic physicochemical properties reminiscent of the proteins to be used for the early diagnosis of AD and treatment of neurodegenerative diseases was discussed by Aliev et al. (2019).

A neuro-biosensor system for determination of CSF levels of α-Syn based on a AuNPs-polyglutamic acid-modified indium tin oxide (ITO) showing a limit of detection of 0.135 pg/ml was characterized with great reproducibility, potency, long storage stability, and regeneration capacity (Karaboga and Sezginturk 2019). AuNPs sensitized ZnO nanorods arrays for DA electrochemical sensing, which were successfully applied to the determination of DA in human urine with satisfied recoveries (95.3–111.3%) and precision (1.1–8.4% of relative standard deviation), were designed by Zhou et al. (2018). By modifying an electrode with reduced GO (rGO) sheet-AuNPs complexes, an electrochemical sensor capable to determine DA and ascorbic acid in a mixture within a range of 0.1–100 μM was obtained, showing a limit of detection of 0.098 μM in the presence of 400 μM ascorbic acid in which π–π interaction of the rGO sheet contributed to improved sensor selectivity, while improved sensitivity was connected with great conductivity and large surface area of rGO sheet-AuNPs (Park et al. 2017). An electrochemical nanobiosensor for early detection of PD based on the quantification of circulating biomarker, miR-195, in which exfoliated GO and Au nanowires were used to modify the surface of screen-printed carbon electrode, showed good selectivity for target miRNA over nonspecific oligonucleotides and the sensitivity of the biosensing with 2.9 fM detection limit (Aghili et al. 2018). Using a tau antibody and an aptamer specific to tau-381 as the recognition element and cysteamine-stabilized AuNPs for signal amplification, Shui et al. (2018a) developed a novel aptamer-antibody sandwich assay based on an electrochemical biosensor for the detection of tau-381 in human serum, showing a limit of detection of 0.42 pM for tau-381. This assay could represent valuable tool in diagnosing AD at the early stages of the disease. A methodology for the specific identification of amyloid fibrils using chiroptical effects in plasmonic NPs utilizing intense chiral response driven by strong dipolar coupling in helical Au nanorod arrangements, which allow to detect amyloid fibrils down to nanomolar concentrations, was reported by Kumar et al. (2018c). A shape-code nanoplasmonic biosensor for multiplex detection of AD biomarkers consisting of only AuNPs and antibody based on the platform through distinct localized surface plasmon resonance depending on shapes of AuNPs was able to achieve a detection limit of 34.9 fM for $A\beta_{1\text{-}40}$, 26 fM for $A\beta_{1\text{-}42}$, and 23.6 fM for tau protein under physiological conditions (Kim et al. 2018). Hong et al. (2009) used a colorimetric detection system consisting of AuNPs coated with SOD-1 for fast diagnosis of ALS. An AgNP-modified ITO electrode covered by GO for the enhanced electrochemical detection of DA was designed by Shin et al. (2017).

By conjugation of the organic fluorescent probe CYDAC(16) with lanthanide-doped upconversion NPs, a composite material providing a ratiometric signal based on an upconversion luminescence at 660 and 800 nm was developed showing good sensitivity and selectivity for Cu^{2+} ions, overdose of which is associated with dis-

eases such as Wilson's disease, Parkinson's disease, and Alzheimer's disease, and this nanocomposite was found to be suitable for the detection of Cu^{2+} in vitro and in vivo (Shi et al. 2019). A novel ratiometric electrochemical biosensor for the dual determination of Cu^{2+} and $A\beta_{1\text{-}42}$ based on a 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonate) and poly(diallyldimethylammonium chloride)-bi functionalized SWCNTs composite with the detection limits of 0.04 μM for Cu^{2+} and 0.5 ng/mL for $A\beta_{1\text{-}42}$, respectively, was designed by Yu et al. (2018).

Direct electrochemical reduction of self-assembled, ex-situ synthesized magnetic hematite (α-Fe_2O_3) anchored GO to reduced GO (α-Fe_2O_3@rGO) on a glassy carbon electrode (GCE) was used for selective detection of DA, whereby α-Fe_2O_3@rGO-modified GCE showed approximately 2.2- and 5-fold higher catalytic activity toward oxidation of DA than α-Fe_2O_3@GO and other modified electrodes, respectively (Mathew et al. 2018). Eight-channel alternating-current magnetosusceptometer of antibody-functionalized magnetic NPs for high-throughput and ultra-high-sensitivity immunoassay to measure three biomarkers of AD, namely $A\beta_{40}$, $A\beta_{42}$, and tau protein, per human specimen, which was developed by Chieh et al. (2018), could be utilized for clinical high-throughput screening of AD. Yang et al. (2017) developed an assay for total tau protein estimation in human plasma based on immunomagnetic reduction (IMR) involving the use of antibody-functionalized magnetic NPs to specifically label target biomarkers and showing the limit of detection related to tau protein 0.026 pg/ml, suggesting that the IMR plasma tau assay would be useful to screen for prevalent neurodegenerative diseases. Magnetic core-plasmonic shell NP-attached hybrid GO-based multifunctional nanoplatform for highly selective magnetic separation of AD biomarkers (Aβ and tau protein), which could be used for "fingerprint" identification of these biomarkers using surface-enhanced Raman spectroscopy at 100 fg/ml level, was developed by Demeritte et al. (2015). As a promising tool for noninvasive in vivo detection of Aβ plaques, sialic acid decorated bovine serum albumin magnetic NPs were recommended by Nasr et al. (2018). A nanoconjugate composed of magnetic NPs bound to an anticholesterol antibody, appropriate to detect the abnormal deposits of cholesterol observed in senile plaques in AD by magnetic resonance imaging, which could provide early information on the onset and progression of AD, was designed by Fernandez-Cabada and Ramos-Gomez (2019).

Recent developments of molecular imprinted polymer-based strategies for the determination of DA in the period 2010–2018 were summarized by Zaidi (2018). An organic field-effect transistor-type nonenzyme biosensor fabricated using PtNP-decorated rGO for ultrasensitive and selective DA detection showing good stability and high sensitivity to very low DA concentrations (100×10^{-18} M) was developed by Oh et al. (2017). A sensitive biosensor for DA detection by differential pulse voltammetry fabricated by electrodepositing PdNPs onto self-supporting nanoporous Au wire showed a broad detection range of 1–220 μM and a low detection limit up to 1 μM (Yi et al. 2017). Aziz et al. (2019) reported a heterostacked nanocomposite prepared by self-stacking of exfoliated positively charged nanosheets of NiAl LDH with negatively charged monolayers of graphene for sensitive detection of DA released from live cells as a diagnostic tool at early stages of PD. Ionic liquid/gra-

phene quantum dot-modified carbon paste electrode was designed as highly sensitive voltammetric sensor for LD determination in the presence of serotonin (Sanati et al. 2017). A disposable flexible *Morpho menelaus*-based wearable sensor integrated with a microfluidic system (in which the structural characteristics of the *M. menelaus* wings up layer are combined with SiO_2 NPs to form a heterostructure) and electronic networks was reported to be suitable for biochemical-physiological hybrid monitoring of neurodegenerative diseases (He et al. 2018).

4.7 Conclusion

The ideal case scenario for the field would be a concomitant progress in the understanding of the pathophysiology of neurodegenerative disorders and development of diagnostic biomarkers. The former would facilitate the design of drugs targeted at crucial steps in the genesis and progression of proteinopathies, while the latter would allow accurate diagnosis, and thus correct enrolment and stratification of patients in clinical trials. Smart drug delivery systems would facilitate the entry of compounds into the CNS and their targeting to key locations in the CNS, or even specific cell populations. Development of accurate theragnostic biomarkers is also necessary to allow the detection of efficacy signals early in clinical development, following the "fail early" philosophy to avoid seeing inefficacious drugs fail only in phase 3 development, as is commonly the case in dementia research (Cummings 2018). Similarly, further development of clinical outcome assessment tools is also necessary, to avoid missing efficacy signals that are actually present by using outdated measures (Hobart et al. 2013).

These developments would give rise to a truly personalized medicine in the field of neurodegenerative disorders, comprising accurate diagnosis of the underlying CNS condition, followed by drugs targeted with surgical precision at the site and type of proteinopathy.

At the level of society and the healthcare system, improvements in care for patients with dementia are urgently necessary, both in capacity and in scope. Even when the first generation of efficacious disease-modifying therapies is developed, these will likely not fully halt the progression of the disease, and improved care models will need to be developed and implemented. Once therapies are able to halt the progression of (or even prevent) neurodegeneration, life expectancy will increase, as it did with advances in the treatment of oncological and cardiovascular disorders, and new challenges arise.

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Chapter 5
Application of Mycogenic Nanoparticles Against Neurodegenerative Diseases

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Abstract Metallic nanoparticles biosynthesis studies such as gold, silver, selenium, iron, metal oxides, and others, by microorganisms, especially fungi, have received great interest from the international scientific community because it is an ecological alternative. This chapter aims to evaluate recent advances in biosynthesis of fungal nanoparticles, with emphasis on physicochemical properties, bioactivity, and applications. Recent advances in biotechnology have provided interesting tools for the controlled synthesis of nanoparticles, using fungi as biotechnological factories. Due to the great diversity of polysaccharide enzymes and polysaccharide structures synthesized by fungi, it makes this kingdom very promising for the nanoparticles biosynthesis. The synthesized nanoparticles present different physicochemical properties, as they depend on biochemical parameters related to the biosynthesis process, besides the conditions used during the process, such as temperature, pH and substrate concentration. Metal nanoparticles associated with

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polysaccharides and proteins; depict bioactivity related to treatment of diseases such as neurodegenerative diseases. These nanoparticles are ecologically friendly and hence can be applied in medicine.

Keywords Biosynthesis · Biotechnology · Fungal · Metallic nanoparticles · Neurodegenerative diseases · Medicine

5.1 Introduction

The study of Fungi has become a very important field in recent years, and is well known for production of secondary metabolites, which control various infectious diseases. In this trend, nanotechnology has emerged as a potential tool for similar applications. Biogenic synthesis of nanoparticles is gaining consideration due to their economic, sustainable, resource-efficient, simple, and ecological nature (Prasad 2017a, b).

Nanotechnology deals with the manufacture of nanoscale materials (1–100 nm). This technology is applicable to the multisectional area because the characteristics of nanoparticles (NPs) are numerous. One of the sub-branches of nanotechnology is "Myconanotechnology" (Rai et al. 2015; Rao et al. 2017a, b) to elucidate fungal-mediated nanofabrication. Nanofabrication is also possible through physical methods. The significance of these methods is decreased over time as biogenic nanosynthesis manifested by bacteria, actinomycetes, fungi, algae, plants and plant parts proved superior in many ways (Rao et al. 2017a, b).

Neurodegenerative diseases are those that leave the patient more debilitated, because it affects his thought, the skilled movements, the feelings, the cognitive, and the memory. Currently, neurodegenerative diseases are the fourth leading cause of death in the developed world after heart disease, cancer, and stroke (Spuch et al. 2012). Each year, more than ten million people worldwide suffer from these diseases. It is estimated that this number will increase by 20% over the next decade, as the aging population grows and lives longer. Many similarities link these diseases to each other at sub-cellular and molecular levels, and discovering such similarities and signaling pathways could offer hope for therapeutic advances that improve many diseases simultaneously (Spuch et al. 2012).

In recent years, there is tremendous growth of research and applications in the field of nanotechnology. There is growing optimism that nanotechnology applied to biomedicine will bring significant advances in the diagnosis and treatment of diseases. The advent of nanotechnology may provide a solution to overcome future diagnosis and new neurotherapeutic challenges for neurodegenerative diseases (Spuch et al. 2012).

Brain disorders treatment is the major challenge due to a variety of formidable barriers to effective and persistent delivery of therapeutic compounds. From various perspectives, the brain is a good challenge for drug delivery. First, an analysis of nonphysical degenerative diseases will increase with an aging population. Second, the blood brain barrier is well known as the body's best guardian over exogenous

substances (Dietrich et al. 2008a, b). Current treatments often have secondary effects that can be more devastating than the disease. The use of engineering in biomedical research has affected human welfare by opening new possibilities in the development of novel types of therapeutic tools. The research on biomaterial engineering and the development of various nanoparticle-based formulations have potential medical use (Gunawardena 2013).

Synthesized nanoparticles by fungi present different physicochemical properties, as they depend on biochemical parameters related to the biosynthesis process, besides the conditions used during the process, such as temperature, pH and substrate concentration. Metallic nanoparticles associated with polysaccharides and proteins and their bioactivity related to diseases treatment such as neurodegenerative diseases are considered in this chapter. These nanoparticles are ecologically friendly and they can be applied in medicine.

5.2 Promising Fungi for Production of Nanoparticles

Fungi kingdom consists of more than 1.6 million species, diversified in the most varied ecological niches, colonizing from the mainland to hostile environments such as deserts, hydrothermal vents and frozen environments such as Antarctica. Like all living beings on the planet, sex genes dominate the evolution of fungi, which determines much of their morphological and biochemical characteristics (James 2015).

Generally, all fungi have a cell wall composed of carbohydrate macromolecules, such as chitin and beta glucans, as well as proteins and glycoproteins. Fungal cells grow as tubular filaments called hyphae, which are coenocytic or septate. The growth of hyphae radially forms a multicellular network called mycelium. Other fungi, however, do not form radial mycelium; therefore, they grow as single cells (unicellular), and reproduce by budding or fission, as is the case of yeasts. The fungal mycelium is the interface site of most bioactivities and a site of intense enzymatic activity (Aydin et al. 2017; Sakamoto 2018).

Fungi are recognized for their ability to possess and use enzymatic tools. It has already been of interest to the international scientific community since the discovery of penicillin in the mid-second world war that fungi have different abilities from other species in nature. In contrast to other living organisms, these eukaryotes will evolve to survive biochemical recycling (Heitman 2015; Halbwachs et al. 2016). It means that these microorganisms are armed with all the enzymatic arsenals, which allows the broadest and most comprehensive biochemical specialization of nature. These complex networked systems, made up of specialized cells, use the enzymatic arsenal to specifically speed up various reactions and modifications in molecular arrangements (Halbwachs et al. 2016).

Researchers around the world glimpse the tools that fungi possess; knowledge of their mechanisms and their peculiarities has helped in the development of new fields of study, technological development, and biomedical applications. The fungi are

described as important molecular factories, which under suitable conditions can be used to produce proteins, carbohydrates, phenolic compounds, vaccines and in the development of monomolecular technologies such as nanoparticles and nanocomposites (Fariq et al. 2017). Studies carried out over the last 18 years (Table 5.1) have shown that fungi, as biotechnological factories, form nanoparticles of the most varied metals and metal oxides, especially filamentous fungi, because they have an enzymatic system capable of producing nanoparticles in the most varied morphological sizes.

The most promising fungi in the development of green technologies for nanoparticle production are those with enzymes capable of efficiently reducing metal ions and metal oxides. In this context, the fungi have varying intracellular and extracellular enzymes capable of acting on metals, reducing them to more stable forms, producing monodisperse nanoparticles with various sizes, morphologies, and geometries. The great advantage of using these microorganisms for the synthesis of nanoparticles is associated with sustainable, rapid, and controlled production. Thus, it can be extended to industrial scales with minimal use of toxic chemicals (Singh et al. 2016).

Fungi have an advantage in relation to other species of microorganisms producing nanoparticles, especially due to the presence of hyphae. As discussed earlier, hyphae are the interface of biochemical reactions in fungi. The production of nanoparticles in bioreactor systems using fungi to reduce metal ions is improved due to the large amount of biomass produced in these systems (Arkowitz and Bassilana 2015; Chen et al. 2018). The yield in nanoparticles is considerably higher when compared to processes using bacteria, algae, and plants. In addition, various species of filamentous fungi such as *Verticillium luteoalbum*, *Collitotrichum* sp., *Fusarium oxysporum*, *Asperigillus oryzae*, *Trichoderma viride*, etc. have been reported as promising species for nanoparticle production, with yields higher than those of other microorganisms (Singh et al. 2016). In addition to the already mentioned filamentous fungi, other species such as superior fungi, producers of fruit bodies have also been studied for the production of nanoparticles.

Nanoparticles production using higher fungi is considered of good quality, since these fungi have diverse enzymatic complexes. The production of enzymes depend on several factors, more especially the composition of nutrients in a culture medium. Thus, the optimization of laccase production conditions using solid-state fermentation of *Pleurotus ostreatus* resulted in enzymes with high catalytic activity. The enzyme was used in the synthesis of gold nanoparticles, proving the catalytic efficiency of the *Pleurotus ostreatus lacasse* (El-Batal et al. 2015). Other basidiomycetes, such as *Ganoderma enigmaticum* and *Trametes* sp., were used to produce silver nanoparticles in submerged culture. The particles were characterized as spherical in a rounded shape, well dispersed without agglomeration. The synthesis of enzymes by the two fungi was efficient in reducing metals to produce nanoparticles, which also have antimicrobial activity (Riddin et al. 2010).

Fungi highlighted above, both in Table 5.1 and in the text, are considered promising due to their morphological, sexual, genomic, and enzymatic characteristics. All these characteristics can be modified and improved by modern techniques of

Table 5.1 Promising fungi for nanoparticle biosynthesis

Fungi	Type of nanoparticles	Size	Shape	References
Fungals				
Fusarium oxysporum Schlecht. Em. Snyder & Hansen	Au	20–40 nm	Spherical, triangular	Mukherjee et al. (2001, 2002)
Fusarium oxysporum Schlecht. Em. Snyder & Hansen	Ag	5–50 nm	–	Senapati et al. (2004)
Fusarium oxysporum Schlecht. Em. Snyder & Hansen	Au–ag	8–14 nm	–	Senapati et al. (2005)
Fusarium semitectum Berk. & Ravenel	Ag	10–60 nm	Spherical	Basavaraja et al. (2008)
Fusarium solani (USM-3799) (Mart.) Sacc.	Ag	16.23 nm	Spherical	Ingle et al. (2009)
Fusarium oxysporum Schlecht. Em. Snyder & Hansen	CdS	5–20 nm	Monodisperse	Ahmad et al. (2002)
Fusarium oxysporum Schlecht. Em. Snyder & Hansen	Silica	5–15 nm	Quasi-spherical	Bansal et al. (2005)
Fusarium oxysporum Schlecht. Em. Snyder & Hansen	Titanium	6–13 nm	Spherical	Bansal et al. (2005)
Fusarium oxysporum Schlecht. Em. Snyder & Hansen	Zirconia	3–11 nm	Quasi-spherical	Bansal et al. (2004)
Fusarium oxysporum Schlecht. Em. Snyder & Hansen *Verticillium* sp. Nees	Magnetite	20–50 nm	Quasi-spherical	Bharde et al. (2006)
Fusarium oxysporum Schlecht. Emen. Snyder & Hansen	CdSe	9–15 nm	Spherical	Kumar et al. (2007)
Aspergillus oryzae var. *viridis*	Au	10–60 nm	Hexagon	Binupriya et al. (2010)
Aspergillus fumigatus Fresenius	Ag	5–25 nm,	Monodispersed	Bhainsa (2006), Prabhu et al. (2009)
Aspergillus flavus Johann Heinrich Friedrich link	Ag	8.92 nm	–	Vigneshwaran Ashtaputre et al. (2007)
Aspergillus niger van Tieghem	Ag	20 nm	Spherical	Gade et al. (2008)
Aspergillus terreus Thom	Zinc oxide	54.8–82.6 nm	Spherical	Baskar et al. (2013)

(continued)

Table 5.1 (continued)

Fungi	Type of nanoparticles	Size	Shape	References
Colletotrichum spp.	Au	20–40 nm	Spherical	Shankar et al. (2003)
Trichothecium spp.Link.	Au	5–200 nm	Triangle, hexagonal	Ahmad et al. (2005)
Trichoderma viride Pers.	Ag	5–40 nm	–	Fayaz et al. (2010)
Verticillium luteoalbum (link) subram.	Au	10 nm	Spherical	Gericke and Pinches (2006)
Phanerochaete chrysosporium Burdsall	Ag	5–200 nm	Pyramidal	Vigneshwaran et al. (2006)
*Volvariella volvacea (*Bulliard ex Fri) singer	Ag andAu–ag	15 nm and 20–150 nm,	Spherical and hexagonal	Daizy (2009)
Cladosporium cladosporioides (Fresen.) G.A. de Vries	Ag	10–100 nm	Spherical	Balaj et al. (2009)
Penicillium brevicompactum WA2315 Dierckx	Ag	58.35–17.88 nm	–	Shaligram et al. (2009)
Penicillium fellutanum Biourge	Ag	5–25 nm	Spherical	Kathiresan et al. (2009)
Phoma glomerata (Corda) Wollen w. & Hochapfel	Ag	60–80 nm	Spherical	Birla et al. (2009)
Neurospora crassa Shear & B.O. Dodge	Ag, au, bimetallic silver and gold	>100 nm	Quasi-spherical	Castro-Longoria et al. (2011)
Yeasts				
Saccharomyces cerevisiae Meyen ex E.C. Hansen	TiO_2	12 nm	Spherical	Jha et al. (2009a)
Saccharomyces cerevisiae Meyen ex E.C. Hansen	Amorphous iron phosphate,	50–200 nm	Spherical	He et al. (2009)
Saccharomyces cerevisiae Meyen ex E.C. Hansen	Sb_2O_3	–	–	Jha et al. (2009b)
*Yarrowia lipolytica*3589 (Wick., Kurtzman & Herman) van der Walt &Arx	Au	15 nm	Hexagonal	Agnihotri et al. (2009)
P. jadinii (Sartory, R. Sartory, Weill & J. Mey.) Kurtzman	Au	<100 nm	Spherical	Gericke and Pinches (2006)
Yarrowia lipolytica NCYC 789	Ag	15 nm	Spherical	Apte et al. (2013)
Extremophilic yeast	Ag, au	Silver, 20; Gold, 30–100 nm	Irregular	Mourato et al. (2011)
Candida utilis NCIM 3469	Ag	20–80 nm	Spherical	Waghmare et al. (2015)

Source: author

biosynthetic biology (Steenkamp et al. 2018). The development of projects with molecular tools for biotechnological application in fungi has guaranteed significant advances in the control and regulation of the expression of proteins and enzymes. The genetic manipulation of these microorganisms is made by special techniques such as advanced promoters and terminators, which allows the control of the expression pathways of genes responsible for the production of specific enzymes (Chae et al. 2017). Another important tool of biosynthetic biology is CRISPR/Cas9, used for gene editing (insertion and deletion of genes), without the need for efficient markers. The development in CRISPR/Cas9, has contributed to the advance in the production of microorganisms more efficient in the production of nanoparticles. Gene editing can be done in the genome simultaneously, using linear DNA, which eliminates cloning steps, facilitating the modification of the metabolic pathways of interest (Mehrotra et al. 2017).

As demonstrated, the future of the production of nanoparticles using microorganisms, especially fungi, depends on the construction of genomic libraries for breeding and editing of genes that are more efficient in the production of reducing enzymes. Several fungi are considered promising for the production of nanoparticles, especially filaments and basidiomycetes, due to the characteristics of their enzymatic arsenals. In the next topic the detailed mechanisms of biosynthesis of nanoparticles by fungi, in addition to some protocols of synthesis will be discussed in detail.

5.3 Advantages of Fungal Nanoparticles Biosynthesis

The biosynthesis of nanoparticles using fungi as biosynthetic factories is quite characterized in the literature, and it is known that the general mechanisms for producing nanoparticles involves the biorreduction of the metal through extracts rich in enzymes. Thus, plants and microorganisms are used for enzymatic reduction of metal salts, leading to the formation of nanoparticles of the most varied nanometric sizes, morphology, and bioactivities. Nanoparticles synthesized using the microbial bioreduction method are more stable and nontoxic, ideal for industrial applications, mainly pharmaceutical (Manivasagan et al. 2016).

General biosynthesis of fungal nanoparticles mechanisms is known; as previously mentioned, the reduction of metal ions through enzymatic mechanisms could occur via intracellular and extracellular means (Sathiyabama and Parthasarathy 2016). However, the details of the pathways and routes of biosynthesis are not yet adequately clarified, mainly because each microorganism has peculiar and unique complexities in its metabolism, which cannot be generalized. Since, it is difficult to demonstrate all the biochemical routes for the synthesis of nanoparticles, the following subtopics should detail the main sets of mechanisms in model organisms, and may elucidate some of the pathways of biosynthesis.

5.3.1 *Intracellular Biosynthesis of Nanoparticles*

The intracellular biosynthesis route requires the chemical recognition by biomacromolecules of the cell wall and the reducing force of the cell to stabilize and displace metallic ions into the cell cytoplasm, or to trap macromolecules in the cell membrane itself (Nasreen and Hulkoti 2014; Patil and Kim 2017). Based on these two principles, when the salt containing metal ions is dissolved in an aqueous solution, the positively charged metal ions are dispersed in the solution. The energy dissipated by the formation of ions in solution increases the degree of disorder of the extracellular system, which contributes to the exchange of free energy in the system. These phenomena help provide an ideal way for the cell wall to chemically recognize ions. The interaction between the positively charged metal ions in solution and the cell wall composed of proteins and enzymes that have specific groups with negative charge is done through electrostatic interactions (Manivasagan et al. 2016).

The ions trapped in the cell wall or in the cytoplasm are then reduced to form small nuclei, later forming nanoparticles of the most varied sizes and morphologies. The intracellular nanoparticle biosynthesis needs to perform a series of reduction reactions, being possible only through a large energy investment. Several reactions coupled in the biosynthesis system (anabolism) inside the cells help to directly channel the chemical energy derived from the oxidative catabolism of NADH. The transfer of protons and electrons between the NADH and another molecule is of high energy, so this intermediate is considered a reducing force charger in enzymatic biosynthesis (Mohanpuria et al. 2008; Golinska et al. 2014). Figure 5.1 shows a simplified scheme of intracellular nanoparticle biosynthesis remedied by fungal metabolism.

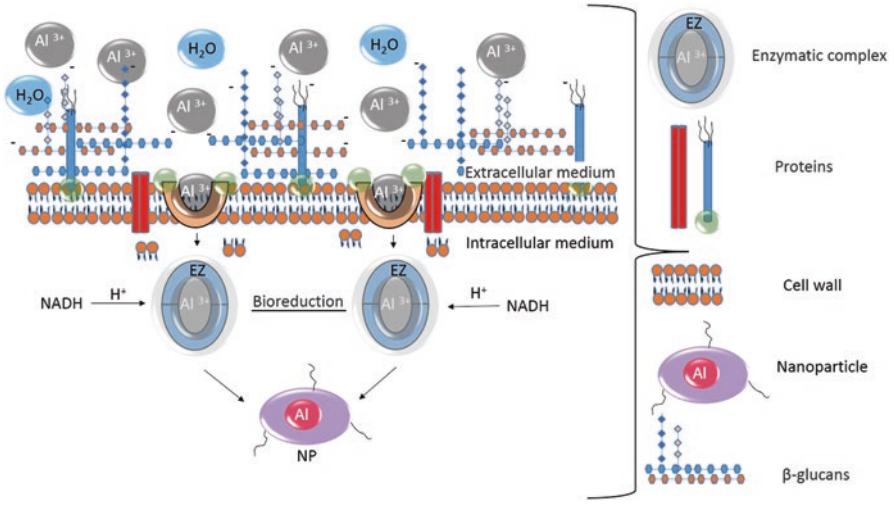

Fig. 5.1 Simplified mechanism of intracellular biosynthesis of aluminum nanoparticle remediated by fungal metabolism

5.3.2 Extracellular Biosynthesis of Nanoparticles

The route of extracellular biosynthesis depends on the chemical recognition of enzymes in the extracellular medium and on its reducing ability. The overall mechanism is more energy-efficient compared to the intracellular mechanism. Since the step of chemical displacement and intracellular reduction are not required, the entire complex of recognition and reduction reactions is conducted outside the cellular environment, or even close to the cell wall. This system of reactions depends on the energy exchange mediated by coenzymes (NAD and NADH), which are the reducing force of the system (Mohanpuria et al. 2008; Golinska et al. 2014).

It is already documented that the nitrate reductase enzyme participates in the biosynthesis of metallic nanoparticles by reducing the ions to more stable unit formulas (Balakumaran et al. 2016). The mechanism of this step is not yet clearly elucidated, but it is known that the reaction depends on selectively stabilized transition states (Balakumaran et al. 2016). Based on these fundamentals we can demonstrate some selective steps of the process of extracellular nanoparticle biosynthesis.

After the solubilization of the salt containing the metal ions in the fungus culture solution, the positively charged metal ions are dispersed in the solution. The energy dissipated by the formation of ions in solution increases the degree of disorder of the extracellular system, which contributes to the exchange of free energy in the system. This step also occurs in intracellular biosynthesis. However, the solution ions are selectively captured by the enzyme nitrate reductase that is in the aqueous solution. The enzyme acts by two recognition pathways. First is to increase the concentration of substrate at the catalytic site. It then guides the conformation of the molecules in a correct orientation so that the reaction is processed correctly (Golinska et al. 2014).

The enzyme nitrate reductase receives energetic help from the NADH coenzyme that transfers electrons to the catalytic reaction site. During the catalytic reaction, the substrate (metallic ions) undergoes several geometric changes and distribution of its electrons before forming the nanoparticles. This step is crucial because the free energy of these intermediate forms, especially of the more unstable forms (transition state), are the ones that most contribute to the speed of the reduction reaction (Iravani et al. 2014; Schrofel et al. 2014). Finally, the metal ions are reduced to small nuclei forming stable nanoparticles. The entire mechanism described in the text is summarized in Fig. 5.2.

5.3.3 General Protocols for Nanoparticle Biosynthesis

Biosynthesis of nanoparticles using fungi has been reported using various protocols. These include the use of microbial biomass (active biomass), cell supernatant, cell free supernatant and biomass extracts. However, it is noteworthy that the extracellular synthesis of metal nanoparticles is more efficient and less costly, due to the

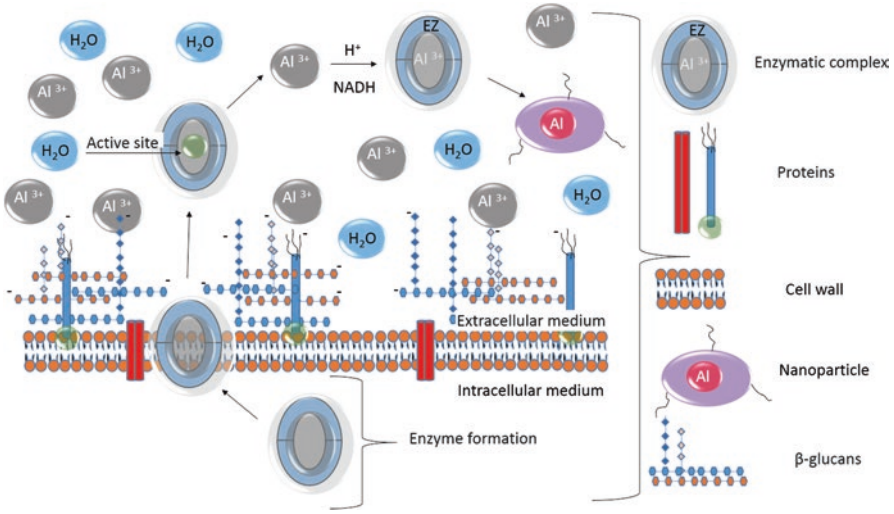

Fig. 5.2 General mechanism of extracellular nanoparticle biosynthesis

simple steps to recover the nanoparticles (Shah et al. 2015). In contrast, intracellular biosynthesis requires more steps to recover the nanoparticles. Especially cell lysis stage, washing of microbial cells for the recovery of nanoparticles and centrifugation to remove cellular debris (Gupta and Bector 2013). Table 5.2, summarizes some protocols for biosynthesis of nanoparticles by fungi.

Among the various nanoparticle biosynthesis protocols, extracellular biosynthesis is the most reported in the literature of the last 18 years (Table 5.2); the nanoparticles produced by these protocols are more monodisperse, with varying sizes and shapes. Based on the literature produced in the last 18 years, we summarize the protocols of synthesis and recovery of nanoparticles in the toolboxes. Box 5.1 summarizes the experimental steps for extracellular nanoparticle production, while Box 5.2 summarizes the experimental steps for intracellular nanoparticle production.

Although nanoparticle biosynthesis has several advantages over the physicochemical mechanisms of conventional synthesis, the polydispersity of formed nanoparticles remains a challenge. Recent studies (Table 5.2) have contributed to establish optimized conditions for nanoparticle biosynthesis. A stable system is considered indispensable for the production of nanoparticles with homogeneous and controlled size and morphologies. The control of the shape and size of the nanoparticles depends on the growth restriction of the microorganisms and also on the changes in the biomolecules produced during the fermentation stage. Thus, nanoparticles biosynthesized by the macro-fungus *Ganoderma* spp. were improved by the optimization of culture conditions (pH, temperature, salt concentration, aeration, incubation period, irradiation, and mixing ratio) (Gurunathan et al. 2014). Specific parameters such as temperature have a significant influence on the kinetics of the enzymatic metal ion reduction reaction for nanoparticles. For example, high temperatures (without inactivating the enzyme) help in the nanoparticle biosynthe-

Table 5.2 Protocols for biosynthesis of fungal nanoparticles

Fungi	Type of nanoparticles	Type of biosynthesis	Reduction condition	Size	References
Verticillium (AAT-TS-4)	Ag	Intracellular	Active biomass	25 nm	Mukherjee et al. (2001)
Candida sp.VITDKGB	Ag	Extracellular	Supernatant	87 nm	Kumar et al. (2011)
Penicillium sp.1–208	Au	Extracellular	Active cell filtering	45 nm	Du et al. (2011)
Penicillium sp.1–208	Au	Intracellular	Active biomass	50 nm	Du et al. (2011)
Penicillium brevicompactum KCCM 60390	Au	Extracellular	Sobrenadante	25–60 nm	Mishra et al. (2011)
Penicillium brevicompactum KCCM 60390	Au	Extracellular	Active cell filtering	20–60 nm	Mishra et al. (2011)
Magnusiomyces ingens LH-F1	Au	Extracellular	Active biomass	10–80 nm	Zhang et al. (2016)
Yarrowia lipolytica NCIM3589	Au	Intracellular	Biomassa ativa	–	Pimprikar et al. (2009)
Aspergillus terreus	Ag	Extracellular	Sobrenadante	1–20 nm	Li et al. (2012)
Aspergillus terreus	Ag	Intracellular	Biomassa ativa	–	Li et al. (2012)
Aspergillus sydowii	Au	Intracellular	Biomassa ativa	8,7–15 nm	Vala (2014)

Box 5.1

In the extracellular biosynthesis, the fungi are cultured for periods between 1 and 4 days, under agitation and optimal conditions of cultivation (pH, temperature, agitation, oxygen flow, nutritional composition, etc.). After the period of growth and microbial metabolism, the broth rich in primary and secondary metabolites (enzymes, polysaccharides, proteins, phenolic compounds, etc.) is separated from the biomass by centrifugation. The obtained supernatant is used for nanoparticle biosynthesis. A sterile solution containing a metal salt is filtered and incubated with the broth under controlled conditions. Finally, the change in the color of the culture broth indicates the formation of nanoparticles. For example, for silver nanoparticles, the color changes to dark-brown, while gold nanoparticles change from red to a strong purple color. To recover the nanoparticles, simply centrifuge the culture broth. This process can be done differentially to selectively separate the nanoparticles.

Box 5.2
For intracellular biosynthesis of metallic nanoparticles, the fungi are cultivated for various periods until the formation of hyphae pellets in the culture solution. The biomass formed is collected by centrifugation, washed with sterilized water, and then dissolved in a sterile filtered solution containing metal salt. The solution is incubated with the fungal biomass under aseptic conditions, with the controlled physicochemical parameters of culture. Nanoparticle biosynthesis is monitored by visual color change in the culture medium. After the incubation period, the fungal biomass is removed to obtain the nanoparticles from the intracellular environment. Thus, the biomass is exposed to ultrasonic waves under various frequencies to provide cell lysis and release of the nanoparticles. Successive ultrasonic extraction, lavage, and centrifugation steps help to isolate nanoparticles from other cellular components.

sis because it increases the energy of the reaction medium, leaving the enzymes more active and selective (Elango and Roopan 2015). pH is another parameter that influences the rate of biosynthesis of nanoparticles. Different ranges of pH are responsible for varying responses to the efficiency of nanoparticle production. Among the fungi, alkaline pH is ideal for *Isaria fumosorosea* (Banu and Balasubramanian 2014), while acidic pH is excellent for *Fusarium acuminatum* fungus, and neutral pH is ideal for the fungus *Penicillium fellutanum* (Kathiresan et al. 2009).

Control parameters such as mixing ratio of the biological extract and metal salt, salt concentration, incubation time, pH, temperature and aeration should be optimized to produce homogeneous nanoparticles of controlled size and shape. Nanoparticle biosynthesis by fungi can provide a layer of additional coverage to nanoparticles, composed mainly of proteins and polysaccharides (Patra et al. 2015). These mechanisms of biofunctionalization of nanoparticles will be more clearly discussed in the next topic.

5.4 Strategies for Surface Functionalization of Nanoparticles

Current strategies for surface functionalization of nanoparticles are a field of research in constant development. Changes in nanoparticle surfaces are necessary requirements to improve the biochemical recognition surface. Various pharmacological and food applications require that the nanoparticles have colloidal stability, which can be achieved by surface functionalization. This strategy assists in the controlled assembly of structures and the delivery of nanoparticles to a specific target, which is only possible due to the presence of appropriate functional molecules on the surface (Ruckenstein and Li 2005).

Although the strategy of surface functionalization of nanoparticles is necessary in several cases, the mechanism of interaction and the stereochemical effects of the nanoscale in the complex nanoparticles and biomacromolecules are not properly understood. Even though nanoparticles have excellent physical and chemical properties, most have unsuitable surface properties for specific applications. In this way, the strategy of functionalization of the nanoparticles using suitable biomacromolecules can alter the composition of the surface, size, and morphology, leaving the complex more appropriate for specific applications (Fu et al. 2006; Bobo et al. 2016).

Nanoparticle surface modification is necessary to reduce the surface energy responsible for the agglomeration of nanoparticles. Therefore, functionalization coats the surface of the nanoparticles with a protective layer which prevents agglomeration, increasing dispersivity, and validity (Bhol et al. 2004). Nanoparticle surface functionalization requires specific functional groups that can interact with various biomolecules that function as a chemical recognition interface that is compatible with real biological systems (Sperling and Parak 2010).

Several approaches are available for functionalization of metallic nanoparticles. However, the widely employed methods are the addition of nanoparticles to vitreous surfaces, incorporation of bivalent binder compounds, deposition of particles on structured surfaces, and use of biomolecules as binders (Lee et al. 2017). Several biomolecules (lipids, vitamins, sugars, polysaccharides, DNA, peptides, and proteins) act as functionalizing agents, since most have some active functional group (carboxylic acid, alcohol, phosphate, primary amine, and thiol group), which can bind the surface of nanoparticles (Gref et al. 2003; Jablonska-Trypuc et al. 2016).

5.5 Bio-Functionalization of Nanoparticles Mediated by Biomacromolecules During Fungal Biosynthesis

Fungal biosynthesis of nanoparticles is a promising strategy for functionalizing the wall of nanoparticles. As discussed in previous sessions on nanoparticle biosynthesis, both the intracellular and extracellular mechanisms are efficient metal ion modifiers. It has also been demonstrated that secondary and primary metals form a protective coating on nanoparticles, which helps in functionalization and stability.

During the biosynthesis of gold and silver nanoparticles, the reduction of the metal ions through the extracellular enzymatic pathway produces dispersed nanoparticles. During the biosynthesis, several exopolysaccharides tend to bind to the surface of the nanoparticles as shown in Fig. 5.3. These exopolysaccharides are excreted in response to changes in the pH of the culture medium, and most of these biopolymers have biological activities such as antitumor, antioxidant and neuroprotective (Huang et al. 2016; Chen and Huang 2018a, b).

Exopolysaccharides produced by fungi during the fermentation step for nanoparticle biosynthesis are dispersed in the solution together with the enzymes responsible for biorreduction. After the reduction of the metal ions, the production of

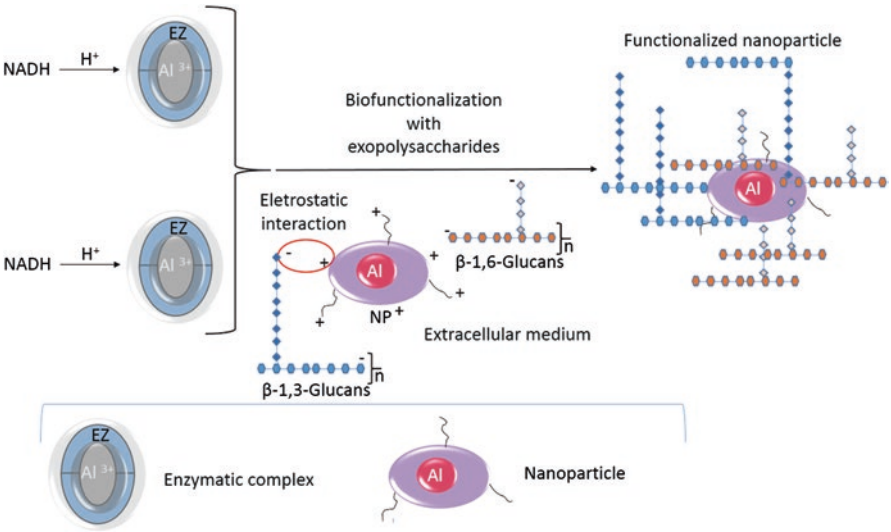

Fig. 5.3 Exopolysaccharides adsorption on the nanoparticles surface

nanoparticles is affected by the presence of biomolecules. The nanoparticles are encapsulated by the exopolysaccharides (Fig. 5.3), which agglomerate in extended conformations, with the ramifications exposed to the external environment (Chen and Huang 2018a, b).

The nanoparticles' surface biofunctionalization also occurs through the intracellular route, mainly by proteins. Proteins are a special class of biomacromolecules, composed mainly of amino acids linked together by peptide bonds (between an amino group and another carboxylic acid); the side chains composed mainly of amino acid residues are the functional groups that can bind to the surface of the nanoparticles (Aryal et al. 2006).

Therefore, the characterization of the interphase between nanoparticles and protein is necessary to understand their physicochemical and conformational properties, as well as to direct the interactions between nanoparticles and proteins. A variety of binding mechanisms between nanoparticles and proteins have been described, especially in conventional synthesis systems (Kathiravan et al. 2005; Murawala et al. 2009). However, these same mechanisms can be hypothetically adapted to explain the formation of nanoparticles with proteins during the intracellular bison. Basically, the mechanisms can be due in two types (covalent and noncovalent bond), according to Fig. 5.4.

In cases where nanoparticles are functionalized by functional groups such as primary amines, thiol surface groups, and carboxylic acid groups, nanoparticles conjugated to amide, ester, and bisulfide linkages are formed. The major proteins that are bioconjugated by the covalent mechanism are immunoglobulins and serum albumin, which have cysteine residues available for surface coupling (Shen et al. 2008).

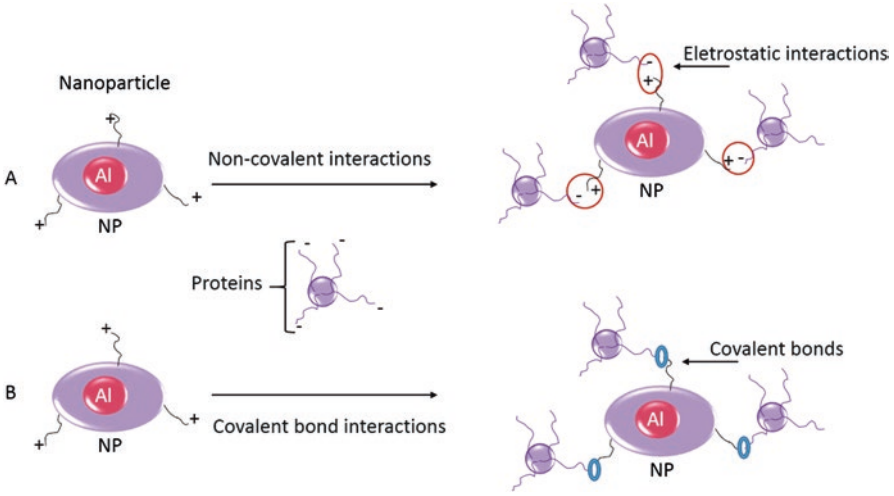

Fig. 5.4 Mechanisms of binding between nanoparticles and proteins. (**a**) noncovalent interactions; (**b**) interactions by covalent binding

Noncovalent interactions are formed by the physical adsorption process, that is, it occurs when the binder (protein) interacts with the surface of the nanoparticles by means of electrostatic forces, hydrogen bonding, and hydrophobic interactions, in addition to the steric modification that stabilizes to a new more appropriate conformation to the surface of the nanoparticles. In these cases of interaction, biomolecules bind directly to nanoparticles through additional binder exchange reactions. As a result of the coating of the nanoparticles by individual biomacromolecules, the surface of the nanoparticles is stabilized and steric repulsion inhibits the agglomeration of the nanoparticles (Shen et al. 2008; Murawala et al. 2009).

Biofunctionalized nanoparticle biosynthesis is a rapidly evolving research area, and is based on a multidisciplinary dataset. Here, we briefly report the formation of supramolecular complexes derived from the bioconjugation of nanoparticles with biomacromolecules, especially exopolysaccharides and proteins. The electrostatic and conformational properties of conjugation have also been reported. Microorganisms in general, most notably fungi, produce primary metabolites very efficiently, which can be used to construct stable and biofunctionalized nanostructures.

5.6 Fungal Nanoparticles Applied in Neurodegenerative Diseases

There are few reports available in the literature on anticholinesterase effects related to fungal-produced nanoparticles. Moreover, these reports lead to an understanding of the mechanisms of biological activity and its peculiarities related to the process

of biosynthesis. Silver nanoparticle biosynthesis (AgNPs) using extracts from the endophytic *Cladosporium* sp. demonstrated antioxidant, antidiabetic, and anti-acetylcholinesterase (AChE) effects of nanoparticles. Nanoparticles (AgNPs) interact with AChE proteins, leading to the inhibition of their activity, and the interaction between the nanoparticles and the protein appears to occur by hydrophobic interactions (Popli et al. 2018). Other works with various nanoparticles produced by other means (organic synthesis and plant-mediated biosynthesis) showed similar effects in several neurodegenerative diseases (Shankar et al. 2017; Saratale et al. 2018). The studies highlighted below corroborate the bioactivity of several nanoparticles in the delivery of drugs that can penetrate the blood–brain barrier (BBB) in the central nervous system (CNS) (Kaushik et al. 2018).

Currently, the existing clinical treatments for the fight against neurodegenerative diseases are simply inefficient, due to the drugs not being able to cross the blood–brain barrier (BBB) to the nervous system, being inadequate to affect the progression of the disease, signs of brain damage or degeneration. In view of this scenario, the use of nanotechnology applied to medicine has emerging potential in clinical practice through the use of nanoparticles that present a possible solution to such problems due to their unique physical and chemical properties that can be exploited as administration of nanoscale drug systems, ensuring that the system/drug complex at the nanoscale, arrives and acts preferentially on the selected target (Malam et al. 2009; Gendelman et al. 2015).

Conjugated Odorrana lectin nanoparticles for the pharmacodynamic study in Parkinson's disease following intranasal administration were investigated by Wen et al. (2011). Odorrana lectin (OL) was conjugated to poly (ethylene glycol)–poly (lactic-co-glycolic acid) (PEG-PLGA) nanoparticles. Administration of OL-conjugated nanoparticles (OL-NP) was performed intranasally, and investigated by fluorescence imaging in vivo using DiR as a tracer. In addition, the urocortin peptide (UCN) was used as a macromolecular drug model, incorporated into nanoparticles, and evaluated for its efficiency in hemiparkinsonian rats after intranasal administration by rotational behavior, neurotransmitter determination, and tyrosine hydroxylase test (TH). The results suggest that the change in Odorrana lectin increased the delivery of the nanoparticles in the central nervous system, and consequently increased the therapeutic effects of the loaded nanoparticles of UNC in Parkinson's disease. It was concluded that nanoparticle-Odorrana lectin conjugates (OL-NPs) can be used as vehicles for the administration of intranasal drugs, especially macromolecular drugs in the treatment of central nervous system (CNS) disorders (Wen et al. 2011).

A study on PLG nanoparticles loaded with curcumin conjugated with the Tet-1 peptide to combat Alzheimer's disease was carried out and the emulsion evaporation method for the synthesis of PLGA-curcumin nanoparticles was used. To allow neuronal targeting of the curcumin nanoparticles, the Tet-1 peptide was coupled which has affinity for the neurons and has retrograde transport properties. The results demonstrated that curcumin-encapsulated PLGA nanoparticles have the ability to destroy amyloid aggregates, as well as exhibit antioxidant activity and are not cytotoxic. Thus, PLGA-curcumin nanoparticles have the potential to be used as a drug with several functions in the treatment of Alzheimer's disease (Mathew et al. 2012).

The distribution of brain-directed nanoparticles loaded with Tempol for neurological disorders was studied. Poly (lactide-co-glycolide) (PLGA) nanoparticles were developed using the nanoprecipitation method, which were loaded with Tempol and conjugated to transferrin antibody (OX 26) covalently using the NHS-PEG3500-maleimide crosslinker. The results showed that the nanoparticles obtained adequate size (80–110 nm) for blood–brain barrier penetration (BBB) and sustained drug release behavior. High cellular uptake of antibody-conjugated nanoparticles was also observed in RG2 mouse glioma cells. Therefore, it can be concluded that nanoparticles conjugated with transferrin containing antioxidants present potential application in the treatment of neurodegenerative diseases (Carroll et al. 2010). Figure 5.5 shows a schematic representation of how nanoparticles with drugs cross the blood–brain barrier for drug delivery.

The use of chitosan nanoparticles incorporated with levodopa (CNL) to be administered intranasally in an attempt to increase their bioavailability in the treatment of Parkinson's disease was evaluated. The levodopa-loaded chitosan nanoparticles (CNL) were prepared and incorporated into a thermoreversible gel, using Pluronic PF127 (CNLP gel). The preparation of CNL and CNLP gel was optimized for formulation parameters such as chitosan TPP ratio, drug loading, and pluronic concentration to obtain the desired CNL particle size, gelation temperature, gelation time, and CNLP gel mucoadhesive strength. The results showed that the in vitro release of CNL obeyed the Higuchi kinetic model, whereas the CNLP gel drug followed the Hixson-Crowel model. In vivo results showed maximum recovery of the drug in the brain following intranasal administration of CNL suspension in saline. Thus, it can be concluded that an intranasal route, if well formulated, can be used as an alternative route effective in drug uptake by the brain. Thus, it contributes to the treatment of neurodegenerative diseases (Sharma et al. 2014).

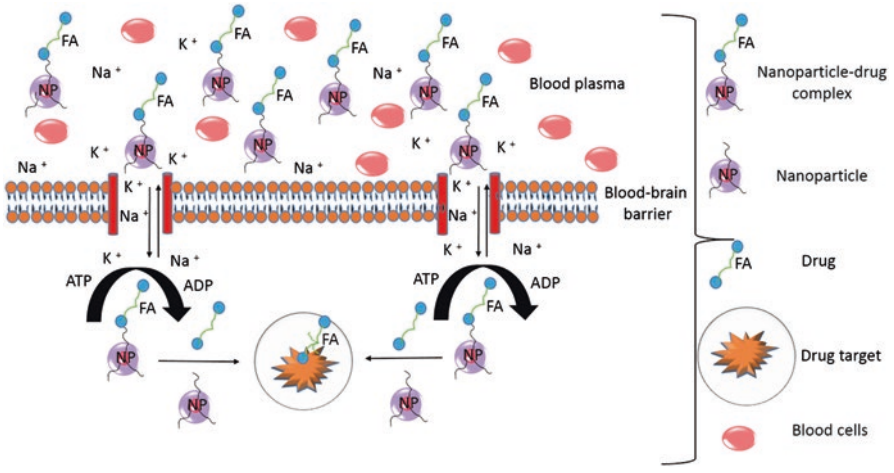

Fig. 5.5 General mechanism of nanoparticles passage through the blood–brain barrier for drug release

5.7 Conclusions

We have seen that protein complexation with nanoparticles has several applications in catalysis, medicine, and the importance of the characterization of the interface between the nanoparticles and protein is necessary for the understanding of its physicochemical and conformational properties. The use of nanotechnology applied to medicine has emerging potential in clinical practice, since the use of nanoparticles present a possible solution to some problems due to their unique physical and chemical properties that can be exploited as administration of nanoscale drug systems, ensuring that the system/drug complex at the nanoscale arrives and acts preferentially on the selected target. Considering the complexity involved in neurodegenerative diseases, there is need for more research studies to consolidate the use of nanotechnology as a treatment for these brain disorders.

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Chapter 6
Nanotechnology-Mediated Nose-to-Brain Drug Delivery for Neurodegenerative Disorders

Chandrakantsing V. Pardeshi, Veena S. Belgamwar, and Sanjay J. Surana

Abstract The blood–brain barrier (BBB) is the major obstacle in the effective delivery of neurotherapeutics to the brain microenvironments. The dense membrane hampers the access of many of the therapeutic drugs to reach to the brain regions in sufficient concentrations, leading to poor patient compliance and discontinuation of the neurotherapy. A direct transport of neurotherapeutics from nose to brain regions along the well-known olfactory and trigeminal nerve pathways seems to be an effective strategy to overcome the BBB, and we propose the direct nose-to-brain drug delivery as an effective strategy for safe and noninvasive administration of the neurotherapeutics. Existence of direct entry point from nose to brain encourages the scientists to explore the opportunities in optimizing therapy of neuroailments. This chapter highlights the diagnosis and therapy of major neurodegenerative disorders (NDs) and developments in the nanotechnological areas for effectively delivering the neurotherapeutics to target the neuronal regions via direct nose-to-brain transport.

Keywords Nanomedicines · Nose-to-brain · Drug delivery · Neurotherapeutics · Neurodegeneration

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6.1 Introduction

The twenty-first century witnessed an unexpected rise in the incidence of central nervous system (CNS) disorders due to an increase in lifespan. Among various CNS disorders, neurodegenerative diseases are the most challenging, and affect ~30 million individuals worldwide (Thomas and Mansoor 2009).

Neurodegenerative disorders (NDs) mark the progressive degeneration of the neurons, usually concerned with death of neuronal cells. Various types of NDs like Alzheimer's (AD), Parkinson's (PD), Prion disease (PrD), Huntington's disease (HD), and Amyotrophic lateral sclerosis (ALS) accompany the neuronal damage in different areas of the brain and spinal cord. Currently, the available therapeutic and diagnostic strategies are inadequate to deter the progression of the brain injury or degeneration for the management of various NDs. The blood–brain barrier (BBB) presents a much difficult hurdle in the transport of therapeutic or diagnostic agents into the CNS (Neuwelt et al. 2008). The nanoneuromedicine offers innovative and promising approaches to treat NDs for which very few treatment options are available (Re et al. 2012).

At present, numerous types of nanomaterials are available with different physicochemical and therapeutic properties. The affirmative merits of the nanomedicines viz., greater chemical or biological substantiality, incorporating capability for hydrophilic as well as hydrophobic cargos, could be useful in the diagnosis or treatment of various NDs. In addition, the nanomedicines can administered from several routes like olfactory, oral, systemic, etc. This review illustrates the variety of characteristics of the nanotechnological approaches involved in the treatment and prognosis of various NDs (Dwivedi et al. 2019).

In order to reach the CNS and effectively treat NDs, a neurotherapeutics must overcome several barriers viz., the blood–brain barrier (BBB) and the blood cerebrospinal fluid barrier. During the last few decades, nose-to-brain delivery for delivering therapeutic drugs to the brain has gained significant attention. There are several pieces of evidence suggesting that the intranasal administration of drugs or biotherapeutic agents (peptides, proteins, gene vectors, and even mesenchymal stem cells) can easily circumvent these barriers in human beings, nonhuman primates, and rodents (Lochhead and Thorne 2012; Lochhead et al. 2015). There is an exclusive anatomical link between the nose and the brain that provides a great opportunity for intranasally administered drugs to rapidly reach the brain parenchyma through several pathways. The olfactory and trigeminal nerves innervating the nasal passages may be the major route for nose-to-brain drug delivery of certain neurotherapeutics (Kozlovskaya et al. 2014). Intranasal delivery has a number of potential advantages such as nose-to-brain delivery to bypass the blood–brain barrier (BBB), which is impermeable to a great number of small molecules and macromolecules (Kozlovskaya et al. 2014).

Direct nose-to-brain drug administration prevents systemic dilution effects and first-pass metabolism, and allows administration of low doses of drugs, which, in turn, reduces peripheral drug exposure, thus lessening the drug toxicity (Pardeshi and

Belgamwar 2013; Kulkarni et al. 2015). Several methods have been explored to enhance the permeation of drugs through the nasal mucosa, including absorption enhancers, and the use of mucoadhesive polymers that prolong the contact time of drugs with the nasal mucosa. The use of nanocarriers has gained much attention, as a novel drug delivery tool, in the treatment of NDs due to their potential for site-specific targeted drug delivery. The nasal route of delivery for delivering neurotherapeutic-loaded novel nanoparticulate colloidal carriers offers effective brain targeting and enhanced drug bioavailability due to minimized systemic exposure when compared to the oral route of delivery (Wen et al. 2011).

This chapter highlights the recent scientific studies concerned with the development of nanomedicine-based pharmaceutical products intended for direct nose-to-brain drug delivery, particularly for the diagnosis or therapy of NDs, particularly for Alzheimer's and Parkinson's disease.

6.2 Nanotechnology-Mediated Nose-To-Brain Drug Delivery for Neurodegenerative Disorders

6.2.1 Alzheimer's Disease

Literature suggests that AD affects more than 24 million people worldwide. Alzheimer's disease leads to onward loss or deterioration of neurons of cortical and hippocampal leads to memory and cognitive dysfunctioning (Goodman et al. 2011). Another neuropathological mark of AD is the presence of neurofibrillary tangles containing hyperphosphorylated tau protein on intraneuronal-paired helical filaments and extracellular plaques of β amyloid peptide (Aβ). Aβ are small fragments of amyloid precursor proteins (APP) containing 39–43 amino acids. Thus, the aggregation of small segments of Aβ (ADDLs, amyloid-β-derived diffusible ligands) is mainly responsible for memory deficits and synaptic damage in AD (Ittner and Gotz 2011).

At present, the available therapies of AD are based on cholinergic progress, particularly on the inhibition of the activities of cholinesterase (AChE and BChE). Although cholinergic inhibitors have greatest benefit for cognitive dysfunction, these are unable to compensate the continuous loss of hippocampal, and cortical neurons. Currently, the treatment of AD depends on acetylcholinesterase (AChE) inhibitors (rivastigmine, donepezil, and galantamine) or NMDAR inhibitor (memantine), mainly administered orally or transdermally, and improves only momentary effects but these are unable to discontinue disease progression (Xiang et al. 2009; Tricco et al. 2013; Molino et al. 2013).

Currently, nanotechnological strategies are employed for enhancing the potency of AD therapies. The nanotechnological devices target Aβ aggregation and fragmentation of APP, not only in CNS but also in blood with the approach of reducing its level in the brain. The reduction of Aβ aggregation and fragmenta-

tion of APP in the brain are known as the 'sink effect' (Matsuoka et al. 2003). Gobbi et al. (2010) developed nanoliposomes (NLs) utilizing phosphatidic acid or cardiolipin (Gobbi et al. 2010). In another investigation, these NLs were found to have greater affinity toward Aβ and diminished the toxicity of these peptides ex vivo (Bereczki et al. 2011).

Nanotechnology attempts more lucrative methods over conventional assays for the estimation of amyloid-beta-derived diffusible ligands or tau protein (pathogenic markers) in human CSF employed in early in vitro diagnosis of AD (Krafft and Klein 2010). The nanotechnological tools were seemed to be much more beneficial in effective imaging of CNS functions, disease states, and also advanced neurosurgical practices. Currently, magnetic resonance imaging (MRI) has appeared as the vital tool for the imaging of brain disorders. Positron emission tomography (PET) imaging is also very potent in the diagnosis of various nervous system disorder mechanisms, including the pathophysiology of AD. In this course, radiolabeled amyloid ligands tracked the pathophysiological process of AD (Jack et al. 2013). While in vivo nanotechnological approaches involve the estimation of Aβ deposits in the brain, the deposition of β amyloid protein in AD transgenic mice was detected from μ-MRI technique which involved Aβ-coupled iron oxide NP, either superparamagnetic or mono-crystalline form (Wadghiri et al. 2013). Roney et al. (2009) synthesized polymeric n-butyl-2-cyanoacrylate NP enclosed with the radiolabeled drug 125I-CQ which has affinity toward amyloid proteins (Roney et al. 2009).

6.2.2 Parkinson's Disease

Parkinson's disease (PD) is a chronic, progressive and age-related neurodegenerative disease affecting seven to ten million people globally (Pardeshi et al. 2013a, b). PD involves the damage to dopaminergic neurons in the substantia nigra-pars compacta and results in difficulties in the movement control. The appearance of Lewy's bodies in the brain of PD patients is the primary sign of this disorder. The Lewy's bodies contain 50–700 nm long filaments of the α-synuclein proteins as cytoplasmic inclusions. Various cellular mechanisms such as ER stress, proteasomal and mitochondrial dysfunctions are behind the neuronal death in PD (Cole and Murphy 2002). The unmet needs and distinct goals in PD are illustrated in Fig. 6.1 (Kulkarni et al. 2015). Also, a scheme comparing healthy brain and PD-affected brain is illustrated in Fig. 6.2 (Kulkarni et al. 2015).

The usual occurrence of PD in late midlife suggested the possible role of aging in pathogenesis of PD as demonstrated by a loss of striatal dopamine and the dopaminergic cells in the substantia nigra. A comprehensive literature search indicated α-synuclein as the most widely investigated genetic determinant for pathogenesis of PD. Along with α-synuclein, several other familial PD-linked genes were subsequently identified, which include Parkin, DJ-1, PINK1, and LRRK2 (Kulkarni et al. 2015).

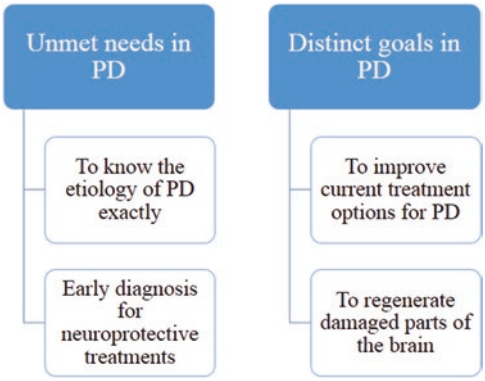

Fig. 6.1 The unmet needs and distinct goals in PD (Adapted from (Kulkarni et al. 2015))

At present, very limited supportive care, management, and cure are available for PD. All these situations slow down the treatment and therapy of PD and alleviate the progression of disease. The available pharmacological treatment and therapy mainly pay its attention on the restoration of dopaminergic neurotransmission (Dwivedi et al. 2019).

The various stages of the symptomatic treatment of PD include monotherapy for early PD, combination therapy for advanced PD, and the combination therapy with different therapeutic agents of different categories for complex PD. For early PD (which is clinically characterized by mild motor disability but without cognitive impairment), monotherapy with MAO-B (monoamine oxidase) inhibitors (selegiline and rasagiline) can be the first-line treatment. In an advanced PD (with mild-to-moderate motor disability without cognitive impairment), treatment can begin with DA agonists (ropinirole, rotigotine, pramipexole, apomorphine, bromocriptine, cabergoline, etc.) along with MAO-B inhibitors. Complex PD (characterized by moderate-to-severe motor disability with cognitive impairment) can be treated with a combination therapy, including catechol-O-methyl transferase (COMT) inhibitors (entacapone, tolcapone, etc.) along with DA precursor (levodopa) (Schapira 2007).

However, the invasive ways can be very competent in providing symptomatic relief, but without any considerable effort to reinstate the root cause of the disease. Thus, the restorative approaches, using cell therapy and neurotrophic support, for dopaminergic nigrostriatal tract may be a very promising practical approach. In the last couple of decades, the cell therapy has acquired a prominent place in the PD treatment by replacement of the degenerating and/or lost dopaminergic neurons of the nigronal striatum (Wijeyekoon and Barker 2009).

Nanotechnological approaches served as a safe and effective strategy over the available conventional treatment lines for the effective management of PD. Tiwari et al. (2013) synthesized nicotine-encapsulated poly(lactic-co-glycolic) acid (PLGA) nanoparticles. At nanosize, the enhanced bioavailability of nicotine with consequent reduction in the oxidative stress and apoptosis led to improved neuroprotective efficacy (Tiwari et al. 2013). Trapani et al. (2011) prepared specific dopamine-coated chitosan NPs. In vivo experiments on rats indicated that

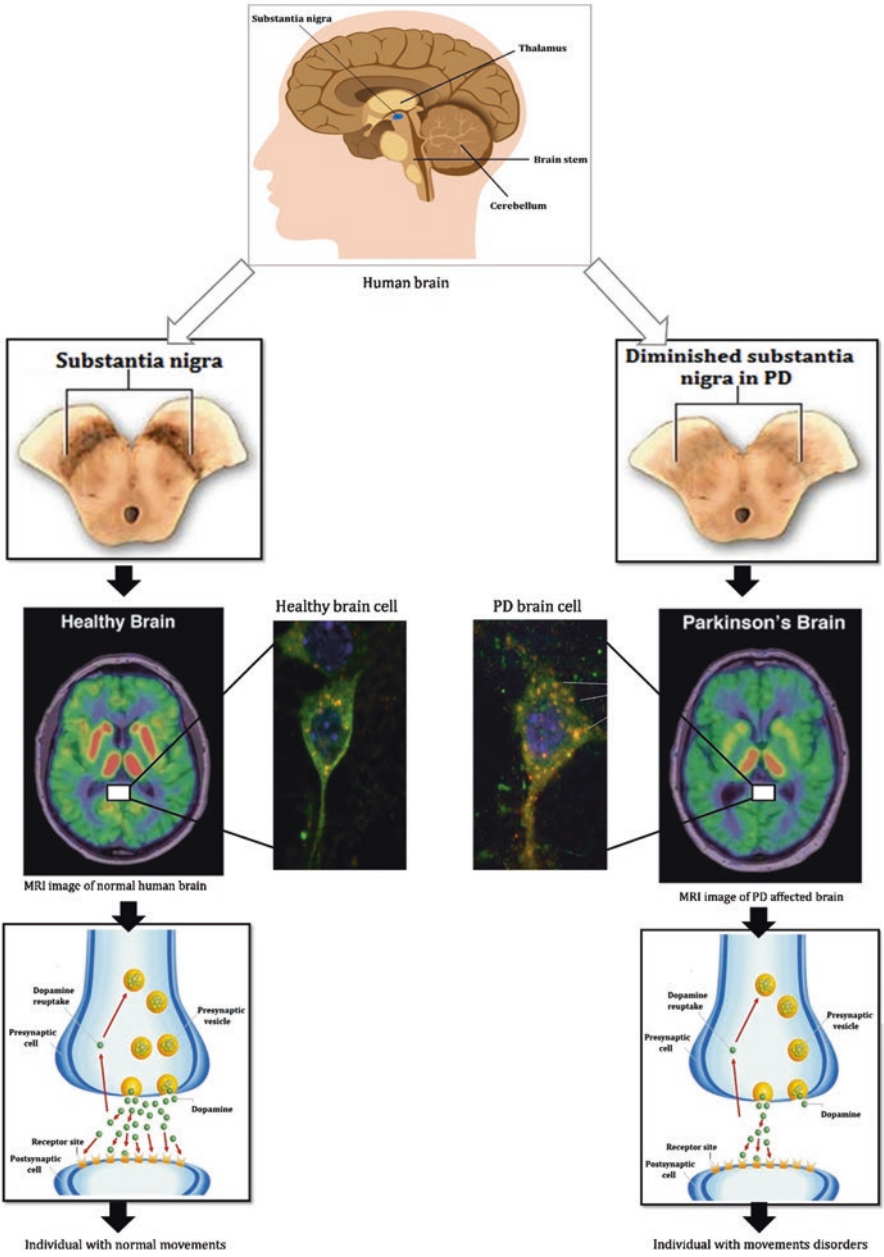

Fig. 6.2 A comparative scheme between healthy brain and PD affected brain. MRI image of PD affected brain shows diminished substantia nigra (SN). Inset view of PD brain cell shows bright reddish dots indicating the garbage disposal lysosomes with α-synuclein. The sick neurons are unable to release DA in sufficient concentration, leading to motor impairments in PD patients (Adapted from (Kulkarni et al. 2015))

the DA-loaded chitosan NPs, on intraperitoneal administration, have less cytotoxicity with greater penetration to the striatum compared to bulk DA alone (Trapani et al. 2011).

There are very few reports available on the in vitro diagnosis of PD. In one report, An et al. (2010) used Au-doped TiO2 nanotubes arrays to design a high-sensitivity photoelectrochemical immunosensor for the detection of α-synuclein, and thereby assist in PD diagnosis. At present, there is no firm datum available reporting the nanotechnology-mediated diagnosis of PD either in preclinical or in clinical stage (An et al. 2010).

6.3 Desirable Properties of Nanoparticles for Nose-To-Brain Drug Delivery

Nanotechnology-mediated controlled and site-specific neurotherapeutic delivery for effective management of PD is imperative and plausible (Sahni et al. 2011). The fabricated NPs offer improved patient compliance due to reduced dose and dosing frequency of administration, compared to drug solution (Wilson et al. 2010). In this regard, the size, ζ-potential, and other surface characteristics are the role-determining properties (Kulkarni et al. 2015).

The Kupffer cells or other phagocytic cells obstruct the biodistribution of neurotherapeutic-loaded NPs of particle size 100 nm or more. On the contrary, hydrophilic drug-loaded NPs of size less than 100 nm have been reported to prevent opsonization with a resultant prolonged duration of action and enhanced site-specific targeting (Banerjee et al. 2002; Vyas and Khar 2002).

Another significant attribute that needs to be focused while formulating NPs is ζ-potential (Hans and Lowman 2002). From a stability standpoint, sufficient electrical potential (greater than ±30 mV) must be retained by NPs to induce static repulsion on reproach (Muller et al. 2001; Tan et al. 2010). Furthermore, ζ-potential also specifies the interaction of NPs with the cells in vivo (Wilson et al. 2008).

In conjunction with these basic traits, NPs must possess the below-mentioned properties, in general, for targeting the brain (Olivier 2005; Pathan et al. 2009): (1) Nontoxic, biocompatible, and biodegradable; (2) Physical stability, in vitro as well as in vivo; (3) Avoidance from reticuloendothelial system (RES) which may prolong the blood circulation time; (4) Scalable and cost-effective manufacturing process; (5) Amenable to small molecules, proteins, and peptides or nucleic acids; (6) Formulation stability, minimal nanoparticle excipient-induced drug alteration (chemical degradation/alteration, protein denaturation); and (7) Controlled drug release profiles.

The nanoparticulate carriers are thought to elicit their action through one of the following principal mechanisms (Pathan et al. 2009):

1. Enhanced retention of NPs formulation for a prolonged time periods on the nasal mucosal surface that would ultimately enhance the delivery of neurotherapeutics across the endothelial cell layer and thereby to the brain regions.
2. Ability of NPs to transiently open the tight junctions of the mucosal epithelium. The neurotherapeutic could then permeate through the tight junctions either in free form or in bound form together with the NPs.
3. Endocytosis or transcytosis of the NPs by the endothelial cells followed by the release of the drug within these cells and its delivery to the brain.

6.4 Pathways and Mechanisms for Nose-To-Brain Delivery of Nanomaterials

Previously, our group (Pardeshi and Belgamwar 2013) had extensively reviewed the pathways and mechanisms for direct nose-to-brain delivery of neurotherapeutics. While the well-defined principal mechanisms underlying intranasal drug delivery to the brain are not entirely investigated so far, a bunch of evidences demonstrated that the important pathways include the nerves (olfactory and trigeminal) connecting the nasal passages to the brain and spinal cord. In addition, pathways involving the cerebrospinal fluid and lymphatic system have been active in the nose-to-brain transport of neurotherapeutic cargos. It is possible that a combination of these pathways is responsible, although one pathway may predominate over other, depending on either the properties of neurotherapeutics, characteristics of the formulation, or the drug delivery device used (Pardeshi and Belgamwar 2013).

6.5 Transport Efficacy of Nanomaterials in Nose-To-Brain Drug Delivery

Nanoparticles (NPs) are able to protect the encapsulated drug from biological and/or chemical degradation, and extracellular transport by P-glycoprotein efflux, and thereby offer an improved nose-to-brain drug delivery approach. This would increase the central nervous system (CNS) availability of drugs (Mistry et al. 2009). It is, however, not demarcated yet whether the drug is being released from the nanocarrier in the nasal cavity where it would be absorbed either transcellularly or paracellularly in the epithelium and transported to CNS, or the nanocarrier itself is transported along olfactory and/or trigeminal nerve pathways into the CNS where the drug is released (Pardeshi and Belgamwar 2013; Mistry et al. 2009).

Various nanocarriers that enable drug delivery systems intended for direct nose-to-brain drug delivery for the treatment of AD and PD are illustrated in Table 6.1.

Table 6.1 Selected reports of various nanotechnology-based nose-to-brain drug delivery systems of neurotherapeutics for NDs

Therapeutic payload	Nanocarriers	Formulation technique	Particle size (nm)	Zeta potential (mV)	Animal model	Therapeutic outcome	References
Nanotechnology-based nose-to-brain drug delivery systems investigated for Parkinson's disease							
Bromocriptine	CS NPs	Ionic gelation	161.3 ± 4.7	40.3 ± 2.78	Swiss albino mice	Reduced catalepsy & reversed akinesia, increased brain uptake, enhanced targeting efficiency	Md et al. (2013)
Ropinirole HCl	CS NPs	Ionic gelation	173.7 ± 2.32	+32.7 ± 1.5	Swiss albino rats	High brain uptake, enhanced mucoadhesion, higher targeting efficiency	Jafarieh et al. (2015)
Levodopa	CS NPs	Ionic gelation	164.5 ± 3.4	28.3	Wistar rats	Improved uptake, avoid degradation of levodopa in peripheral circulation, enhanced residence	Sharma et al. (2014)
bFGF	GNLs	w/w emulsion-freeze drying	143 ± 1.14	−38.2 ± 1.2	Sprague-Dawley rats	Enhanced olfactory uptake, low ciliotoxicity	Zhao et al. (2014)
Ropinirole HCl	SLN	Emulsification-solvent diffusion	98.43 ± 3.3	29.91 ± 2.14	Albino mice	Enhanced stability, reduced dosing frequency	Md et al. (2014)
Ropinirole HCl	PLN	Solvent emulsion-diffusion	66.22 ± 6.2	28.19 ± 3.02	Albino mice	Mild ciliotoxicity, improved stability, reduced dose and dosing frequency	Pardeshi et al. (2013b)
Nanotechnology-based nose-to-brain drug delivery systems investigated for Alzheimer's disease							
Donepezil	Chitosan Nanosuspension	Ionic crosslinking	100–200	NM	Wistar rats	Showed higher percentage of radioactivity per gram in the brain for the donepezil loaded chitosan nanoparticles formulation as compared to donepezil solution.	Md et al. (2014)
Rivastigmine	Chitosan NPs	Ionic gelation	163.7 ± 7.6	38.40 ± 2.85	Wistar rats	Improved bioavailability and enhanced uptake into the brain.	Fazil et al. (2012)
Estradiol	Chitosan NPs	Ionic gelation	269.3 ± 31.6	24.8	Wistar rats	Enhanced retention, high brain uptake	Wang et al. (2008)

NM not mentioned

6.6 Concluding Remarks and Future Outlook

A major hurdle in drug delivery to the brain is the presence of the BBB that restricts the diffusion of drugs from systemic circulation into the CNS even if it is disrupted in certain pathological conditions. This could be the reason that many neurotherapeutic agents failed to stay on the pharmaceutical market because of their inability to achieve sufficient levels in brain through systemic circulation. Intranasal administration offers a practical, safe, convenient, and noninvasive alternative to various conventional and invasive drug delivery techniques as a transport pathway for direct delivery of drugs effectively to the CNS, bypassing the BBB. Nose-to-brain drug delivery is most likely intervened through the olfactory and/or trigeminal nerve pathways. Neuroepithelium is merely the sole part of CNS that is directly exposed to external environment through the nasal cavity. Thus, improved target specificity can be accomplished due to direct passage of drug from the submucosal space of the nose into the CSF compartment of brain.

Nanoneurotechnology, particularly application of nanotechnology for drug delivery in the treatment of NDs, has provided promising answers to the related issues in recent years. There will, probably, be a need for the development of multiple therapeutic strategies that can act via different mechanisms. This would provide optimal treatment for the patients who might be suffering from different causes or severity of NDs.

Delivery of surface-engineered nanocarrier systems through active or passive targeting approach would be desirable for further progress in the field as well. To sum up with an opinion, the nanotechnology-based systems developed for clinical applications for the treatment of NDs should be made available at affordable prices besides being accepted by the pharmaceutical market.

In conclusion, we are optimistic that the nanotechnology-based nose-to-brain drug delivery would eventually become an important adjunct to the therapies offered to the NDs patients in near future.

Conflict of Interest The authors declare no conflict of interest.

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Chapter 7
Nanobiotechnology in Parkinson's Disease

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Abstract Parkinson's disease (PD) is a complex neurodegenerative disorder. It is characterized by a combination of motor and nonmotor symptoms that gradually appear as consequence of the selective loss of dopaminergic neurons in the *substantia nigra pars compacta* and the presence of Lewy bodies and dystrophic neurites, two abnormal structures composed by misfolded α-synuclein. Recent evidences suggest that the toxicity caused by α-synuclein relies on its oligomerization, which precedes the formation of the large α-synuclein aggregates. Several important contributions have been made in the PD field during recent years. However, an early and accurate diagnosis, together with the availability of disease-modifying therapies, still represents a major unmet need. The emergence of nanotechnology has provided new systems like ultra-sensitive biosensors that are able to detect PD-related biomarkers in complex but more accessible biological fluids, and novel MRI agents for contrast enhancement in imaging applications. Nanotechnology could also revolutionize the PD therapeutic pipeline, which is currently focused on the relief of motor symptoms. To date, the efficacy of nanotechnology in PD treatment has been supported by a large number of preclinical studies that have

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encapsulated different drugs in a wide range of nanoscale delivery systems such as nanoparticles, liposomes, exosomes, and quantum dots. In this chapter, we provide an overview of recent advances in the application of nanomedicine to both the diagnosis and treatment of PD. The main challenges anticipated, future perspectives, and the possibility of transferring these studies to future clinical trials are also discussed.

Keywords Parkinson's disease · Nanotechnology · Diagnosis · Treatment · α-synuclein · Neuroprotective therapies

7.1 Introduction

Parkinson's disease (PD) is a complex neurodegenerative disorder clinically characterized by a triad of motor symptoms, including tremor, rigidity, and bradykinesia. PD is the second most common neurodegenerative disease after Alzheimer's disease (AD) (Kalia and Lang 2015), with a prevalence of approximately 0.5–1% among people 65–69 years of age, rising to 1–3% among those aged 80 years and older, and both the prevalence and incidence of PD are expected to increase by more than 30% by 2030 (Nussbaum and Ellis 2003).

In recent years, with the emergence of new genetic, biological, and imaging biomarkers, supported by the neuropathology findings and the clinical variability presented by PD patients, a growing consensus has emerged that PD is a heterogeneous disorder with variable clinicopathological phenotypes (Thenganatt and Jankovic 2014). Several efforts are now being made to introduce novel genetic, imaging, and biochemical biomarkers to define and categorize PD patients into different subgroups of patients who might be more responsive to novel therapies (Chen-Plotkin and Zetterberg 2018).

The etiology and pathogenic mechanisms of PD remain poorly understood, with only a small proportion of PD having a monogenic cause (with an overall prevalence lower than 5%) (Poewe et al. 2017). The majority of cases are idiopathic, and a handful of studies have suggested that these idiopathic cases could be caused by a combination of susceptibility factors and environmental influences, although the relationship between the development of PD and factors such as inflammation, head trauma, diabetes mellitus, and pesticide exposure is unclear (Kouli et al. 2018).

More recently, several studies have suggested that the clinical diagnosis of PD is preceded, even 5–15 years before the onset of the motor symptoms, by a "prodromal" phase when nonmotor symptoms are evident (Poewe et al. 2017). The first nonmotor symptoms that appear are slight depression, constipation, fatigue, sleep disturbance, and hyposmia (Poewe et al. 2017; Kouli et al. 2018). This prodromal

phase of PD has attracted increasing interest in the last few years, since it might be an ideal point for therapeutic intervention.

Pathologically, PD is characterized by loss of dopaminergic neurons in the *substantia nigra pars compacta* (Poewe et al. 2017) and the presence of abnormal cytoplasmic inclusions within neuronal cell bodies, which are immunoreactive for the protein α-synuclein (Spillantini et al. 1997). These pathological protein aggregates are called Lewy bodies (LBs) and are often accompanied by dystrophic neurites (Lewy neurites), which are present in numerous brain nuclei, and which engage an increasing number of brain regions as the disease progresses (Braak et al. 2003). Although the precise role of α-synuclein in PD is not fully understood, the fact that subjects with mutations or multiplications of the α-synuclein gene develop PD indicates that altered α-synuclein expression in neurons could be sufficient to promote nigral degeneration (Singleton and Hardy 2016).

Furthermore, recent studies regarding the role of α-synuclein in PD have made a breakthrough in our understanding of the mechanisms that might contribute to disease progression. On the one hand, several studies have now highlighted that the toxicity caused by α-synuclein relies on its accumulation and oligomerization, which precedes the formation of large α-synuclein aggregates (e.g., Lewy bodies) (Kalia et al. 2013). Another important advance is the emergence of evidence that α-synuclein oligomers can spread from one cell to another in a prion-like manner (Brundin and Melki 2017). According to this prion hypothesis, oligomers or small amounts of fibrillar α -synuclein act as "seeds" which can trigger the conversion of soluble α-synuclein monomers into insoluble α -synuclein aggregates or fibrils. Furthermore, these "seeds" can be released from neurons and taken up by neighboring neurons, astrocytes, or glia, thus inducing the spread of pathologic α-synuclein throughout the nervous system (Brundin et al. 2017).

Taking together all these lines of evidence, identifying the toxic species formed during α-synuclein amyloid formation and establishing how they cause neuronal death remain priorities for the development of novel therapeutic strategies. It is important to address many questions that still remain elusive, including how aggregation is initiated and the role of the cellular environment in the aggregation process, in order to open new therapeutic strategies that could arrest or, ideally, halt PD progression.

7.2 Current Diagnostic Strategies

The ante-mortem diagnosis of PD is based mainly on clinical features. The current clinical criteria define PD as the presence of bradykinesia and at least one additional cardinal motor feature (rigidity or rest tremor), as well as additional supporting and exclusionary criteria (Postuma et al. 2015). Therefore, an accurate medical history and physical examination are fundamental to achieve a correct diagnosis.

An accurate diagnosis of PD is essential for prognosis and therapy, as well as for clinical, pharmacological, and epidemiological studies. However, the diagnosis of

PD remains inadequate, even in specialized Movement Disorder Units, in which the diagnostic accuracy is about 82% (Rizzo et al. 2016).

For this reason, several diagnostic approaches and biomarkers have been introduced to increase diagnostic confidence, and some of these have been included in the recent published diagnostic criteria (Postuma et al. 2015).

7.2.1 Diagnostic Test

Currently, diagnostic tools for PD can be classified into two different categories: biochemical-genetic-based markers and imaging biomarkers.

Several biochemical biomarkers such as α-synuclein, tau, or Aβ (Emamzadeh and Surguchov 2018) have been studied in blood, plasma, serum, and cerebrospinal fluid, but the results reported are inconsistent and consequently, they cannot be considered a real diagnostic test. Genetic tests are usually only considered in subjects with a strong suspicion of a possible genetic cause, in early-onset PD cases, and in subjects with an atypical clinical presentation (Berardelli et al. 2013).

On the other hand, imaging tools are used not only to rule out symptomatic parkinsonism and other degenerative parkinsonism, but also to increase the diagnostic accuracy of PD as supporting findings. Computed Tomography (CT) scan and Magnetic Resonance Imaging (MRI) are not obligatory, but both are recommended, especially when atypical features are present. For example, MRI can show specific findings related to a particular atypical parkinsonism syndrome (APS) (Broski et al. 2014), although the sensitivity levels are low.

Molecular imaging technologies allow the in vivo study of neurotransmitter systems as well as the metabolic abnormalities that occur in different neurodegenerative disorders. 18F-fluorodeoxyglucose (18F-FDG) Positron Emission Tomography ([^{18}F] FDG-PET) is used to visualize regional glucose consumption in the brain. Reasonably specific disease-related patterns have been described in PD and APSs (Tang et al. 2010), but they have not yet been included in the diagnostic criteria for PD. Dopamine transporter (DaT) imaging (DaT-SCAN) is used to visualize presynaptic nigrostriatal dysfunction, so it helps to differentiate between PD and nondegenerative parkinsonism and patients with essential tremor. Unfortunately, it is not useful to discriminate between PD and APSs, or even among APSs, since nigrostriatal degeneration is a common pathological feature of all of them (Xu et al. 2018). Iodine-123–labeled metaiodobenzylguanidine single-photon emission computed tomography ([^{123}I]MIBG-SPECT) is an imaging technique that reflects the integrity of the postganglionic sympathetic nerve terminals, which degenerate in PD and Lewy body dementia patients. Consequently, cardiac [^{123}I]MIBG-SPECT can serve to differentiate these disorders from other APS. However, the false-negative rate is still high in early or prodromal stages of the disease.

7.3 Treatment

There are currently no disease-modifying treatments for PD, and medical management is predominantly focused on controlling motor symptoms. These symptoms occur due to the selective loss of neurons in the *substantia nigra pars compacta*, which lead to a depletion of dopamine in the striatum (Munchau and Bhatia 2000; Jankovic 2008). Thus, the mainstays of PD treatment at present are drugs designed to replace the action of dopamine in the striatum of these patients. This may be achieved through drugs that are metabolized to dopamine, that activate the dopamine receptor, or that prevent the breakdown of endogenous dopamine (AlDakheel et al. 2014). However, as dopamine is not the only neurotransmitter involved in PD, many other drugs are also being used to target specific symptoms, such as depression or dementia. Nowadays, there is no gold standard treatment strategy, with medication being customized for each patient, based on the severity of their symptoms, as well as the side effects that they experience (Munchau and Bhatia 2000; Jankovic 2008; AlDakheel et al. 2014).

When the first motor symptoms appear, there is a preference for the use of dopamine agonists and inhibitors of monoamine oxidase isoform B (MAO-B), one of the main enzymes involved in the breakdown of dopamine [19]. Their utilization relieves motor symptoms in PD patients, and they are used as a levodopa-sparing strategy. These therapies are usually enough to control the symptoms in early disease, but as disease progresses, most patients require a levodopa-based treatment. This treatment is designed to replace the dopamine in the depleted striatum. Levodopa is a dopamine precursor, which, unlike dopamine, is able to cross the blood–brain barrier (BBB) and can be administered as a therapy. But as the disease progresses, PD patients suffer a deterioration of motor state that requires higher levodopa doses and/or an increment in dosing frequency which result in an elevated risk of developing problematic adverse effects, including significant motor complications, like dyskinesias, and severe on–off motor fluctuations. Some strategies used to counteract the adverse effects of high doses of levodopa include fractionation of the dose, and administering levodopa in combination with peripheral inhibitors of DOPA decarboxylase, since some of its associated side effects result from the conversion of levodopa to dopamine outside the central nervous system (CNS) by DOPA decarboxylase (Munchau and Bhatia 2000; Jankovic 2008; AlDakheel et al. 2014). Other options for advanced PD include administration of MAO-B inhibitors such as safinamide, dopamine agonists (pramipexole, ropinirol, and rotigotine), or COMT inhibitors like entacapone or opicapone. Furthermore, in patients who have previously responded well to levodopa, but have developed problematic dyskinesias, deep brain stimulation may be considered, which may allow for the control of motor symptoms on a reduced dose of levodopa (Munchau and Bhatia 2000). In other cases, intestinal levodopa gel infusion and subcutaneous apomorphine infusion significantly reduce motor fluctuations and improve the quality of life of PD patients.

7.3.1 Emerging Treatments

Besides the treatments mentioned above, a number of novel approaches are currently under investigation (Munchau and Bhatia 2000). Among them, one of the most promising strategies is immunotherapies targeting α-synuclein, which are now beginning to enter clinical trials. In a Phase 1 clinical trial, the synthetic vaccine, Affitope PD03A, containing an α-synuclein mimicking peptide, has been tested in 36 patients with early-stage PD, who received the vaccine subcutaneously, and it was found to be very well tolerated, with only mild side effects (NCT02267434, Zahoor et al. 2018). Furthermore, passive immunization using the PRX002 antibody, a humanized monoclonal antibody with which a 96.5% reduction in free serum levels of α-synuclein was observed has also been tested in Phase 1a and Phase 1b clinical trials (NCT02095171, Schenk et al. 2017). No major side effects or toxicity were found, and the antibody has progressed to Phase 2 clinical trials (NCT02157714, Zahoor et al. 2018). Another α-synuclein-based passive immunotherapy led by Biogen, the BIIB-054 antibody, was found to be well tolerated with a satisfactory pharmacokinetic profile (NCT03318523, Brundin et al. 2017).

In addition to new experimental compounds based on targeting α-synuclein, there is also much interest in drug repurposing—the use of drugs that have an established clinical indication—in a new setting. Two existing drugs have now entered clinical trials for PD treatment: the chemotherapy agent, nilotinib, and the glucagon-like peptide-1 receptor agonist, exenatide. Nilotinib is a c-Abl tyrosine kinase inhibitor used in the treatment of chronic myelogenous leukemia. The activity of c-Abl has been found to be enhanced in brain tissue of PD patients, which may enhance the oligomerization of α-synuclein, or reduce the mitochondrial biogenesis (Lonskaya et al. 2014). Nilotinib has been well tolerated by PD patients (Pagan et al. 2016) and has entered a Phase 2a trial in 2017 (Munchau and Bhatia 2000). Similarly, exenatide, an established treatment for type 2 diabetes mellitus, is emerging as a promising therapeutic option for PD. Neuroprotective potential has been seen in preclinical models of the disease (Li et al. 2009) and a recent clinical trial using exenatide (Athauda et al. 2017) reported positive effects on off-medication motor scores in PD patients with no history of type 2 diabetes mellitus.

7.4 Nanobiotechnology as an Emerging Tool

As stated previously, despite the extensive research in progress for more effective antiparkinsonian agents, antioxidants, neurotrophic factors (NFs), and immunotherapies against α-synuclein, there is a great need for innovative and more effective ways to deliver these treatments within the CNS (Rodríguez-Nogales et al. 2016).

Nanobiotechnology has emerged as a powerful tool that could facilitate the CNS delivery of drugs and particles with novel formulations, allowing sustained drug release over time, reducing toxic effects and making it possible to cross the BBB

(Garbayo et al. 2014; Mavridis et al. 2018). One example of the power of nanobiotechnology for changing PD treatment is the case of dopaminergic trophic factors. NFs, especially glial-derived neurotrophic factor (GDNF), have received great attention in PD since GDNF was able to enhance the survival of midbrain dopaminergic neurons in culture (Obeso et al. 2017). On the basis of those observations, and after a series of studies in which gene delivery of GDNF improved the motor symptoms and enhanced nigrostriatal regeneration using PD animal models, one clinical trial testing the safety and efficacy of GDNF following intraventricular delivery in patients with PD was performed. Unfortunately, this trial reported no efficacy (Nutt et al. 2003). Another double-blind trial testing the efficacy of intrastriatal GDNF protein in PD patients was also performed (Lang et al. 2006), but again, the trial did not meet its primary endpoint. After the analysis of these trials and taking together the experimental evidence on the neuroprotective role of GDNF on preclinical models, one conclusion that could be drawn is that GDNF was not delivered in an optimal way, and several attempts have subsequently been made in order to achieve this goal (Whone et al. 2019). Recently, our group has shown the successful brain delivery of encapsulated NFs that promote neuronal survival and reverse PD progression in rodent and nonhuman primates (Garbayo et al. 2009; Garbayo et al. 2011; Garbayo et al. 2016).

The **aim** of this chapter is thus to explore the role of nanobiotechnology in PD, from a diagnostic and a therapeutic point of view. A wide range of applications of nanobiotechnology to PD will be presented, including new diagnostic methods and promising therapeutic targets combined with the newest brain delivery systems that nanobiotechnology has recently provided.

7.5 Nanotechnology for PD Diagnosis

As mentioned before, the lack of diagnostic methods beyond an accurate medical history means that a great effort is needed to develop accurate diagnostic tools that allow an early diagnosis of PD. In this regard, a wide variety of nanomaterials have revolutionized the medical field, offering interesting applications that could improve the diagnosis of this neurodegenerative disorder. For instance, nanoparticles (NPs) can be used as MRI agents for contrast enhancement in imaging applications that can generate new noninvasive diagnostic approaches. Additionally, NPs can also be used to develop minimally invasive biosensors that are able to detect PD biomarkers with high specificity and sensitivity. For instance, the combination of NPs with immunomagnetic reduction (IMR) assays would allow the detection of biomarkers at lower concentrations than traditional methods. In this section, we provide an overview of novel nanosystems for an improved diagnosis of PD underlining the most promising strategies (Fig. 7.1). Figure 7.1 depicts the most relevant nanotechnology-based approaches for PD diagnosis.

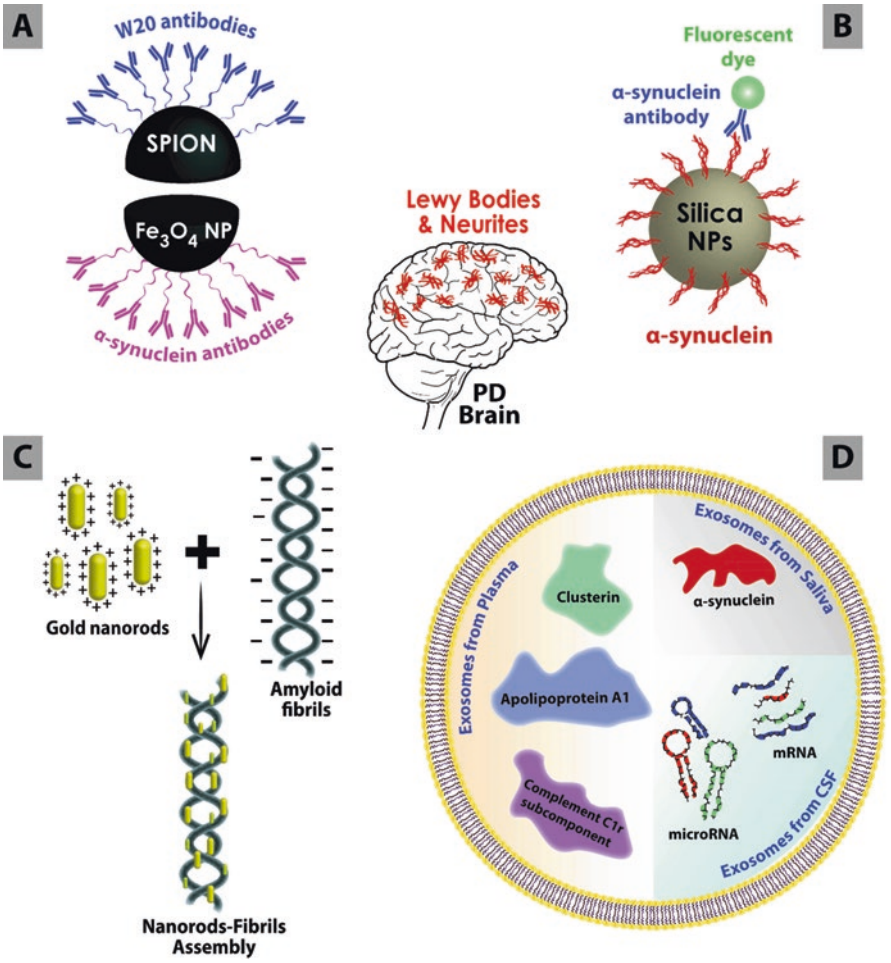

Fig. 7.1 Nanosystems investigated for PD diagnosis: (**a**) Functionalized hybrid nanoparticles to detect PD biomarkers by IMR. (**b**) Silica NPs functionalized with α-synuclein as a standard calibration in a α-synuclein immunoquantification method in CSF. (**c**) Detection of amyloid fibrils with gold nanorods. (**d**) Analysis of natural exosomes from plasma, cerebrospinal fluid and saliva as new tools for PD diagnosis

7.5.1 Hybrid Nanosystems for PD Diagnosis

Hybrid nanosystems, defined as the combination of organic and inorganic compounds in a unique nanocarrier, constitute a new generation of multifunctional NPs which have superior biological and structural properties. Hybrid nanosystems offer immense opportunities to improve diagnostic tests for this neurodegenerative disorder.

7.5.1.1 Antibody-Based Diagnostics

A novel approach to detect PD biomarkers is based on the design of functionalized NPs. Basically, NPs could be conjugated with specific antibodies that bind to their target with high affinity. To date, these strategies have been tested using MRI and with biological samples using IMR.

In Vivo Detection

This approach combines functionalized NPs with MRI for the detection of targets located within the CNS. Superparamagnetic iron oxide NPs (SPIONs) were conjugated with a specific anti-oligomer conformational antibody (W20) (Fig. 7.1a). This hybrid nanosystem has shown promising results for the development of an MRI-based diagnostic tool (Liu et al. 2019). W20-SPIONs were intravenously administered in a transgenic mouse model of PD reaching the area where the amyloid deposits were located and producing a MRI signal that is able to differentiate between PD animals and healthy controls. These results support the capacity of these NPs to specifically recognize oligomers, which are the toxic species responsible for initiating the pathological processes in different neurodegenerative diseases. However, since oligomers are not specific to PD, this method could only be used to diagnose the presence of misfolded proteins in the brain. Consequently, this strategy is an interesting starting point for future lines of research that could focus on the development of nanosystems based on antibodies against presynaptic dopamine transporter or conformational antibodies against the oligomeric forms of α-synuclein.

Detection in Biological Samples

From another perspective, functionalized NPs could be used as reactive for IMR. This technique would allow the quantification of possible PD biomarkers with high sensitivity and specificity, even at low concentrations. Based on this concept, Fe_3O_4 NPs were functionalized with antibodies against α-synuclein to differentiate between two clinical stages: Parkinson disease dementia (PDD) and PD (Fig. 7.1a). Fe_3O_4 functionalized NPs were used to quantify α-synuclein at ultra-low concentrations in human plasma by IMR, and the signal obtained was correlated with the concentration of α-synuclein (Yang et al. 2016b). The results obtained showed that plasma levels of α-synuclein were significantly higher in PDD than in PD patients with normal cognition, while α-synuclein plasma concentration for control subjects was significantly lower than in both groups. This study offers preliminary evidence for applying IMR for PD and PDD diagnosis by assaying plasma α-synuclein levels. Overall, this strategy could be applied for the detection of any potential biomarker in blood, plasma or cerebrospinal fluid (CSF), being a potential diagnostic tool, and also a promising prognosis marker of the disease.

Oligomer-Based Diagnosis

Diagnostic tests based on oligomers constitute a novel strategy that should allow the detection of the specific toxic forms related to PD etiopathogenesis. Nevertheless, the lack of suitable oligomeric standards has limited the accurate quantitation of the absolute α-synuclein oligomer concentration in body fluids. For this reason, the development of standard molecules that mimic the native oligomer is mandatory. Under this condition, a new tool for the validation of diagnostic tests based on oligomers was developed by Hermann et al. In this study, silica NPs were functionalized with α-synuclein and used as a standard calibration in an α-synuclein immunoquantification method in CSF (Fig. 7.1b). The validity of α-synuclein NPs of silica as standard calibration was tested using a surface-based fluorescence intensity distribution analysis assay (sFIDA), which is a technique that allows us to determine the concentration of oligomers of α-synuclein. α-synuclein NPs of silica were developed with a size inside the established ranges of oligomers of α-synuclein and with a high number of epitopes (Herrmann et al. 2017). Hybrid NPs were successfully employed as a standard calibration for the sFIDA assay demonstrating that this standard could be used in any immuno-based method for the quantitation of native α-syn oligomers.

7.5.1.2 Gold Nanorod-Based Diagnosis

Gold nanorods are a promising nanodevice in several medical applications such as diagnostics, bioimaging, and therapy (Taheri et al. 2018). In this regard, the chiroptical effects of gold nanorods have been investigated for the specific identification of amyloid fibrils in PD. The helical nature of amyloid fibrils was used to achieve the assembly of gold nanorods on their surface (Fig. 7.1c). The helical nanorod assembly was able to produce an intense chiroptical activity, which allowed the detection of amyloid fibrils at very low concentration (nanomolar). The detection of amyloid fibrils in PD-affected brain homogenates was demonstrated by the observation of an intense plasmonic chirality, whereas in both monomeric recombinant proteins and brain homogenates from control subjects, the plasmonic chirality was absent or very weak. A biodetection technique was thus successfully applied to human brain homogenates of PD subjects, but the application of this strategy has been limited to the detection of amyloid (nonspecific for PD) and therefore its validity for PD-specific α-synuclein oligomers should be evaluated.

These studies reveal that hybrid nanosystems may be considered as potential tools for PD diagnosis. In this regard, the ability demonstrated by IMR for detecting a low concentration of biomarkers in neurodegenerative parkinsonisms should be highlighted. On the other hand, nanocarriers conjugated with specific antibodies for MRI-based diagnosis provide an encouraging approach that would allow the recog-

nition of specific molecules or structures related to PD (α-synuclein, presynaptic dopamine transporter, and so on). Relevantly, this strategy implies a noninvasive technique, and further studies are required to make the future transfer to the clinical setting possible.

7.5.2 Exosomes for PD Diagnosis

Exosomes are naturally nanosized vesicles that can be released from different cell types, including cells located in the central nervous system (CNS) as oligodendrocytes and neurons. Exosomes have a relevant role in neuronal and glial communications and great potential to promote neuronal regeneration (Izadpanah et al. 2018; Gámez-Valero et al. 2019). In addition, the pathophysiological status of PD patients promotes intracellular changes that could be reflected in exosome components, representing promising biomarkers for PD (Izadpanah et al. 2018; Abdel-Haq 2019). In this arena, the detection and the analysis of neuronal exosomes have been mainly investigated in three biological fluids: human plasma, CSF, and saliva (Kitamura et al. 2018; Ohmichi et al. 2018; Cao et al. 2019) (Fig. 7.1d).

7.5.2.1 Exosomes from Human Plasma

In recent years, the study of exosomes isolated from human plasma has attracted enormous attention for diagnostic applications. In fact, a very recent study isolated and characterized neuron-, astrocyte-, and oligodendrocyte-derived exosomes (NDEs, ADEs, and ODEs, respectively) from human plasma. The plasma levels of NDE and ODE were significantly higher in PD patients compared with healthy controls (Ohmichi et al. 2018). Nevertheless, we should also take into account a possible increase in exosome plasma levels (NDEs, ADEs, and ODEs) in other neurological disorders. Therefore, these biomarkers cannot be considered specific molecules for PD diagnosis, but they may indicate the presence of a global CNS disease. In another example, exosomes were also isolated from plasma of 16 PD patients and their proteomic profile was compared with 8 healthy subjects. Three exosomal proteins (clusterin, complement C1r subcomponent, and apolipoprotein A1) were differentially expressed, revealing a significant decrease compared to healthy subjects (Wu et al. 2017; Izadpanah et al. 2018; Kitamura et al. 2018). The plasmatic quantitation of these exosomal proteins could be used as possible PD biomarker, but before this, the specificity of these exosomal proteins should be demonstrated in other neurodegenerative processes such as atypical parkinsonism or Alzheimer's disease.

7.5.2.2 Exosomes from Cerebrospinal Fluid

Another remarkable approach is based on the study of exosomal miRNA content from CSF. In this sense, miRNAs are very promising candidates as future biomarkers. However, to date, they have not been validated for PD diagnosis. In one example, different profiles of miRNA exosomal levels for PD and AD in neurotrophin signaling and dopaminergic synapse pathways were found. In addition, exosomal mRNA levels were tested, revealing significant differences compared to healthy controls but not between AD and PD subjects (Gui et al. 2015). Regarding exosomal mRNAs, further studies with a larger sample size are required to validate them as diagnostic test for PD.

7.5.2.3 Exosomes from Saliva

The determination of PD biomarkers in salivary exosomes would provide a revolutionary and noninvasive method for PD diagnosis. Recently, the presence of α-synuclein in exosomes isolated from salivary samples has been demonstrated by Cao et al. This study was focused on investigating the presence of exosomes in PD saliva and the evaluation of α-synuclein levels within the isolated exosomes as a potential biomarker for PD, and higher levels of α-synuclein oligomers were found in the exosomes obtained from the saliva of PD patients compared to healthy subjects. Consequently, α-syn oligomers in salivary exosomes could provide relevant information and serve as biomarkers of PD, offering the possibility of developing a noninvasive method for PD diagnosis (Cao et al. 2019).

Collectively, these studies support the hypothesis postulated by many other authors, who have related the content of exosomes secreted by neuronal cells in blood with the health-state of their originating cells/tissues, confirming the potential role of exosomes as PD biomarker sources (Izadpanah et al. 2018; Abdel-Haq 2019). In summary, this constitutes a novel and promising basis on which to develop noninvasive and specific diagnostic methods for the detection of biomarkers (α-synuclein, microRNAs) closely related to the pathophysiological changes observed in PD patients.

7.6 Nanotechnology for PD Treatment

Currently available treatments show a lack of therapeutic options to halt PD progression. This situation has encouraged the search for new therapeutic alternatives with the aim not only to ameliorate the symptoms, but also to reverse the neurodegenerative process. In the face of this paradigm, nanotechnology has emerged as one of the mainstays in the investigation of effective drugs to overcome the barriers against this disease. Figure 7.2 summarizes the most novel nanosystems proposed

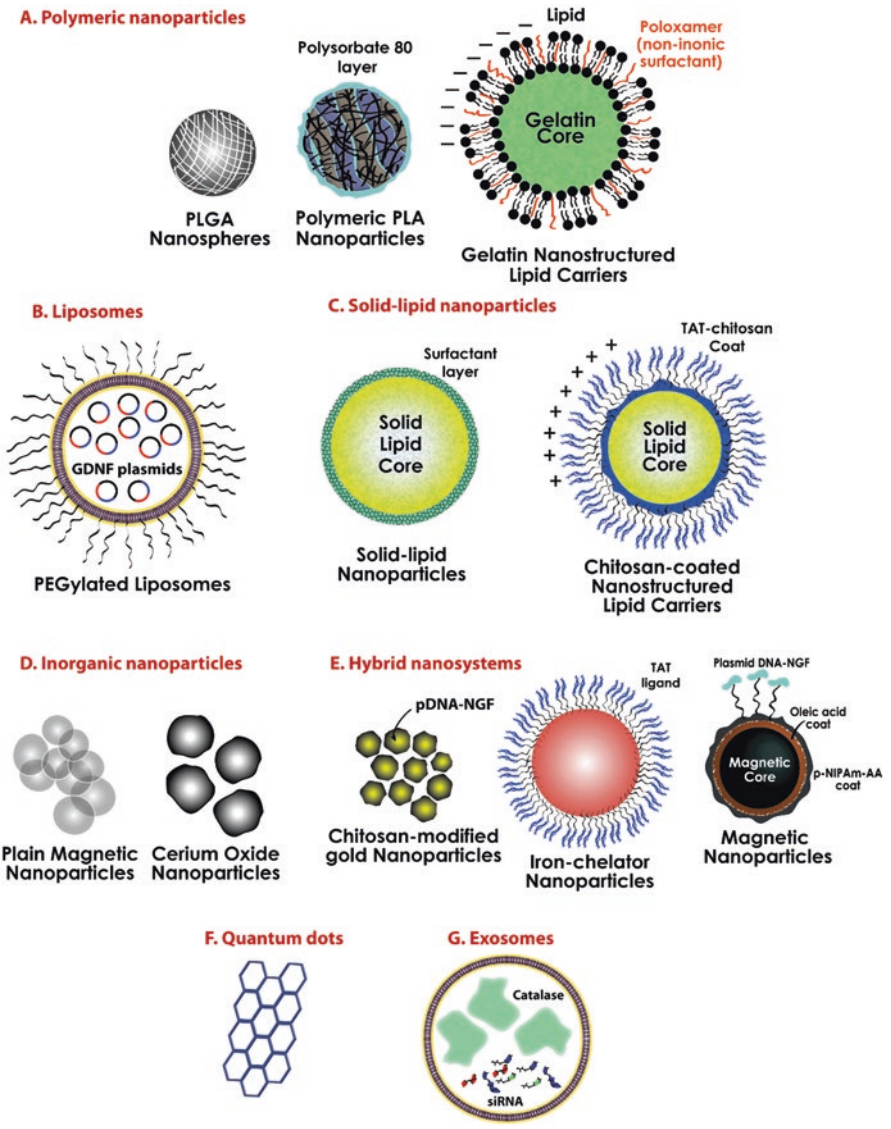

Fig. 7.2 Schematic representation of the most novel nanosystems proposed to improve PD therapeutics. Reprinted and modified with permission (Torres-Ortega et al. 2019): (**a**) PLA, PLGA and Gelatin NPs. (**b**) GDNF-DNA plasmid encapsulated into pegylated liposomes (**c**) Solid-lipid NPs and CTS-coated nanostructured lipid carriers loaded with GDNF. (**d**) Plain magnetic and cerium oxide NPs. (**e**) Gold NPs loading pDNA-nerve growth factor (NGF) and modified by CTS, Iron NPs functionalized with TAT, and plasmid DNA-NGF encapsulated into magnetic NPs coated with oleic acid molecules. (**f**) Graphene quantum dots. (**g**) Catalase and siRNA exosomes

to improve PD therapeutics. In this section, we focus on the most attractive and promising nanobiotechnology approaches developed to fight PD.

7.6.1 Polymeric NPs

Polymeric NPs (Fig. 7.2a) are being extensively investigated to improve the transport of drugs across the blood–brain barrier (BBB) and more specifically, to achieve novel delivery systems for PD therapy. In this regard, polymeric NPs offer the advantage of being prepared with biocompatible and biodegradable materials (Kreuter 2014). Moreover, polymeric NPs have suitable features in terms of simple elaboration and design, and they can be compatible with other strategies such as tissue engineering (Nitta and Numata 2013; El-Say and El-Sawy 2017).

7.6.1.1 Neurotrophic Factor Therapies

A wide variety of drugs have been encapsulated into polymeric NPs. Among them, NFs stand out as one of the most promising groups of therapeutic drugs for PD therapy. In the past, the efficacy of different NFs was examined in preclinical models of PD, showing the protection of dopaminergic neurons and motor recovery (Yasuhara et al. 2005; Tome et al. 2017). However, there are examples demonstrating the necessity for better drug delivery vehicles for their administration. For instance, as previously mentioned, clinical trials examining the efficacy of the free GDNF failed to confirm the previously observed benefits (Nutt et al. 2003; Eslamboli 2005; Lang et al. 2006; Garbayo et al. 2016; Tome et al. 2017; Whone et al. 2019). These results encouraged a large number of researchers to investigate the use of polymeric NPs to improve NF delivery to the brain. In one critical study, a combinatorial therapy using PLGA nanospheres (NS) (Fig. 7.2a) containing GNDF and VEGF was investigated (Herrán et al. 2014). Upon local administration in parkinsonian rats, behavioral tests showed a functional improvement together with a significant recovery of dopaminergic neurons in both the striatum and *substantia nigra pars compacta*. Thus, these results corroborated an important synergistic effect promoted by the local administration of both NFs. Moving forward, the investigation of less invasive routes for the dual administration of these NFs could be interesting for the treatment of early stage or prodromal PD patients.

Alternatively, novel strategies based on the combination of nanomedicine with gene therapy have been explored to deliver GDNF to the brain. One such approach involves the nanoencapsulation of DNA encoding for human GDNF into polymeric particles. These complexes were designed to deliver plasmids to the brain cells and to transfect postmitotic cells. After local administration, a higher number of nigral dopaminergic neurons and an improved motor function were found in 6-hydroxydopamine (6-OHDA) lesioned rats. Furthermore, a partial protection of TH^+ fibers in the lesioned striatum was also reported. These data effectively demon-

strate that synthetic NPs are able to deliver therapeutic genes to the brain, producing a transgenic expression responsible for their neuroprotective effect (Yurek et al. 2017). Another significant example is the design of angiopep-modified NPs to encapsulate GDNF genes. The conjugation of NPs with angiopep facilitated the passage of NPs through the BBB, since angiopep is a specific ligand that is able to bind to a low-density receptor-related lipoprotein overexpressed in the BBB (Huang et al. 2013). Importantly, the intravenous NP administration was able to ameliorate locomotor dysfunction and to restore dopaminergic neurons in the nigrostriatal system, demonstrating that angiopep-conjugated NPs could effectively promote GDNF gene expression in vivo.

Besides these strategies, a noninvasive dual strategy based on magnetic resonance image-guided focused ultrasound (FUS) and brain-penetrating NPs (BPN) has been recently investigated (Fig. 7.3a) (Mead et al. 2017). Previous studies have demonstrated that FUS in conjunction with contrast agent microbubbles (MBs) was able to noninvasively and temporarily open the BBB (Timbie et al. 2015). GDNF-BPN and MBs were intravenously co-injected, leading to an extended and uniform GDNF expression throughout the targeted brain tissue. Relevant levels of GDNF located in the FUS-targeted regions in the striatum of parkinsonian rats were found. As a result, this treatment was able to restore both dopamine levels and dopaminergic neuron density and ameliorate motor dysfunction with no evidence of local or systemic toxicity. Therefore, the development of a minimally invasive gene therapy was achieved, with great capacity to reach therapeutic levels of GDNF in the brain, and its efficacy was demonstrated in animals with severe dopaminergic loss (≈70–80%). This example constitutes a novel, improved strategy to target NFs to specific brain regions, achieving therapeutically effective concentrations with minimal invasiveness.

Recently, another study has explored the potential of gelatin nanostructured lipid carriers (GLNs) for the intranasal delivery of bFGF. Several studies have reported that the presence of NP at the delivery site could be lengthened with negatively charged drugs compared to a neutral drug with similar size and lipophilicity (Elzoghby 2013; Zhao et al. 2016). Taking this into account, GLNs with a strong negative charge of −27.6 ± 1.1 mV were developed. As a consequence, GLNs were efficiently delivered to the striatum, improving nigral dopaminergic neuron survival and attenuating rotational behavior (Zhao et al. 2014). Moreover, these GLNs proved to have superior properties to bFGF gelatin NPs.

7.6.1.2 Antioxidant Therapies

Oxidative stress is the most important cause of mitochondrial DNA disruption, which seems to have huge importance in PD etiology (Olanow 1993). Consequently, great efforts have been focused on the study of antioxidant molecules and the improvement of new ways to design stable and bioavailable formulations for PD. For example, retinoic acid has been demonstrated to have a neuroprotective role (Maden 2007; Esteves et al. 2015). Its encapsulation could improve brain bioavail-

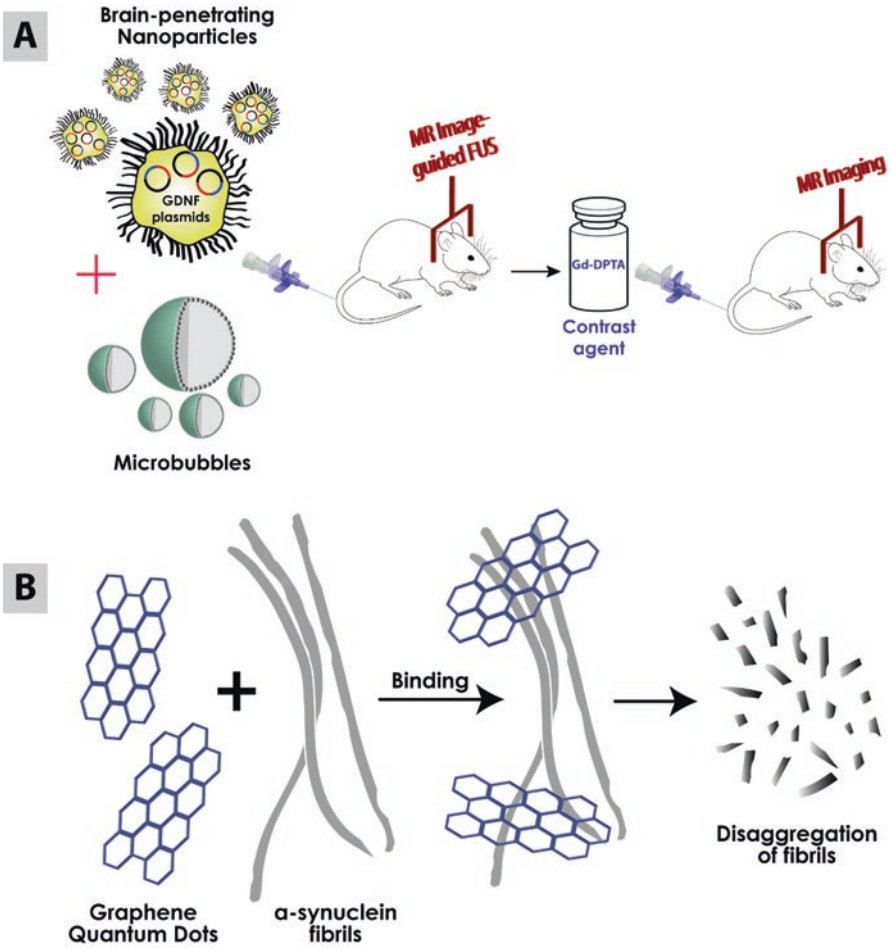

Fig. 7.3 Promising nanotechnology-based approaches for PD treatment: (**a**) Dual therapy based on magnetic resonance image-guided focused ultrasound (FUS) and brain-penetrating GDNF NPs. (**b**) Disaggregation of α-synuclein fibrils using quantum dots

ability and solve some limitations such as its low solubility in water and short in vivo half-life. Under these premises, Esteves et al. designed novel retinoic acid-loaded NPs of around 220 nm for stereotaxic delivery. The effects of these NPs were tested in a mouse PD model, producing a significant reduction in the loss of nigral dopaminergic neurons and an increase in the expression of transcription factors responsible for dopaminergic neuronal specification and survival (Santos et al. 2012; Esteves et al. 2015). Taking these results into account, retinoic acid NPs could be at least a promising prophylactic strategy of neuroprotection for PD.

One other antioxidant drug that has also been nanoencapsulated in polymeric NPs is nicotine. Nicotine has a great permeability to cross the BBB due to its high

liposolubility. However, this molecule requires a carrier that is able to release low doses of nicotine in the brain since high doses would mean toxicity and side effects (Tiwari et al. 2013). To solve this problem, Tiwari et al successfully encapsulated nicotine into PLGA NPs. The recovery of TH-positive neurons and microglial activation was tested and compared with bulk nicotine-treated mice in MPTP model. The intraperitoneal administration of nicotine NPs was able to produce a substantial recovery of TH-positive neurons and a significant reduction of microglial activation compared with bulk nicotine-treated mice (Tiwari et al. 2013). These results suggest that nicotine nanoencapsulation is an interesting way to improve its effectiveness. Unfortunately, the entrapment efficiency of nicotine obtained in this study was very low and more experiments are needed to improve the encapsulation process.

Among the numerous antioxidant compounds, resveratrol is a promising candidate to treat PD (Lu et al. 2008; Jin et al. 2008; He and Yan 2013; Carrizzo et al. 2013). In this regard, Lindner et al. have developed oral resveratrol-loaded polysorbate 80-coated poly(lactide) (PLA) NPs (Fig. 7.2a) to solve its limited oral bioavailability and to investigate its effects in PD (Lin et al. 2010; Rege et al. 2014; da Rocha et al. 2015). The neuroprotective effects of resveratrol NPs were tested in a mouse PD model. An interesting finding of this study was the prevention of the striatal TH decrease induced by MPTP when animals were treated with resveratrol NPs and not with bulk resveratrol (da Rocha et al. 2015).

Beneficial effects in two animal models of PD (rat and zebrafish) have also been reported using an antioxidant and anti-inflammatory medicinal herb named "Schisantherin A" (Li et al. 2014). A stronger neuroprotective effect was achieved on oral administration of this compound in nanocrystals compared to administration of the free drug (Chen et al. 2016). In summary, the formulation in nanocrystal form could effectively improve the oral bioavailability and the brain delivery of Schisantherin A.

7.6.1.3 Other Neuroprotective Therapies

MicroRNAs play a key role in the regulatory process of neurodegenerative disorders such as Alzheimer's and PD (Lim et al. 2010). The combination of microRNA technology with nanotechnology has been proposed as a promising therapeutic strategy for PD treatment. For example, Saraiva et al. designed NPs loaded with miR-124-, a microRNA with pro-neurogenic properties for neural stem cells (Shen and Temple 2009; Lim et al. 2010; Saraiva et al. 2016). These NPs were able to promote not only neurogenesis but also the migration and maturation of new neurons in the damaged striatum of mice treated with 6-OHDA (Saraiva et al. 2016).

The important regulatory role of substance P (SP) (Barker 1991) on the dopaminergic pathway encouraged Zhao et al. to investigate the potential of gelatin NPs (Fig. 7.2a) loaded with SP to treat PD. In this study, intranasal gelatin NPs effectively delivered SP into the brain, improving motor behavior and stimulating the recovery of the neuronal function in damaged areas of the brain. These effects were supported by a notable increase in the number of dopaminergic neurons, a reduction

in glial cell proliferation, inflammatory cell infiltration, and connective tissue hyperplasia in a rat PD model (Zhao et al. 2016). This study could help us to understand the real implications of SP for the survival of dopaminergic neurons and the underlying mechanisms responsible for their therapeutic effects.

In summary, polymeric NPs have been explored as delivery carriers for NFs, antioxidant molecules and miRNA, demonstrating great promise for PD treatment in PD animal models. Notably, studies using polymeric NPs have provided interesting evidence not only for the local administration of bioactive compounds, but also for other routes of administration such as intraperitoneal, intravenous, oral, and intranasal.

7.6.2 Liposomes for PD Treatment

In the past few years, increasing attention has been paid to the role of liposomes in PD (Fig. 7.2b). Compared to other nanocarriers, liposomes are more easily modifiable to achieve effective delivery across the BBB (Agrawal et al. 2017; Ross et al. 2018). Relevantly, these systems might also be developed to establish specific interactions with molecular targets involved in PD and could be loaded with not only hydrophilic but also lipophilic/hydrophobic drugs, offering several approaches to address PD therapy.

7.6.2.1 Neurotrophic Factor Therapies

In a recent study, GDNF-DNA plasmid (Fig. 7.2b) was encapsulated into liposomes and conjugated with microbubbles to be guided by FUS. A significant restoration of motor function together with an increase of TH-positive neurons and DA synthesis by cellular GDNF transduction was reported following intravenous administration in a MPTP PD model (Lin et al. 2016). In another interesting study, Yue and coworkers studied GDNF plasmid gene delivery using liposomes. In this case, ultrasound-triggered effects of the microbubbles coupled to GDNF plasmid-loaded PEGylated liposomes (PLs-GDNF-MBs) were investigated in a rat PD model. Collectively, behavioral dysfunctions were improved in parkinsonian animals by intravenous PLs-GDNF-MBs administration using MRI-guided FUS. Specifically, the number of apomorphine-induced rotations, climbing pole, and suspension tests were significantly ameliorated with PLs-GDNF-MBs. Likewise, the treatment was able to promote GDNF and Nurr1 expression levels, leading to protection from the neuronal loss, which was also reflected in the rescue of TH and dopamine transporter (DAT) expressions (Yue et al. 2018a). Later on, the same research group improved these neuroprotective effects, loading liposomes with GDNF and Nurr1

plasmids (Yue et al. 2018b). This evidence positions liposomes as an effective carrier to encapsulate and deliver plasmid genes related to PD to the brain. Lately, the administration of intranasal liposomes has been investigated in a rat PD model. In this case, liposomes were loaded with GDNF, obtaining very high entrapment efficiency (>90%), together with an improvement in the nose-to-brain delivery of GDNF. In consequence, liposomes ameliorated motor impairments and GDNF neuroprotective effects in 6-OHDA rat model of PD (Migliore et al. 2014). This approach therefore offers an alternative route for the administration of NFs as GDNF and constitutes a noninvasive strategy that bypasses the BBB and delivers therapeutic drugs directly to the brain (Torres-Ortega et al. 2019).

In addition to GDNF, the administration of basic fibroblast growth factor (bFGF) using liposomes has also been investigated by several authors. In one study, a small ubiquitin-related modifier (SUMO) was incorporated in recombinant human FGF20 (rhFGF20) and loaded into liposomes. The treatment was administered into the tail vein, and FUS was used to transport these liposomes across the BBB. The main objective of this study was to test the efficacy of this combinational method to improve the solubility of rhFGF20, the efficacy of FUS to transport liposomes across the BBB, and to produce a neuroprotective effect in a 6-OHDA-lesioned rat model of PD. An improved solubility and more efficient delivery of SUMO-rhFGF20 liposomes to the brain were reported. In addition, the treatment increased dopaminergic neuron survival and reduced apomorphine-induced contralateral rotations in 6-OHDA rats (Niu et al. 2018). In another approach, the intranasal delivery of bFGF in liposomal formulation was found to regulate phosphorylated tau and to induce neuroprotective effects. Tau protein is involved in many neurodegenerative disorders, and recent studies support the presence of tau pathology in PD (Freeman et al. 2017; Zhang et al. 2018). The aggregation and deposition of this protein would contribute to the presence of intraneuronal tau inclusion and neurofibrillary tangles in PD (Freeman et al. 2017; Zhang et al. 2018). Consequently, neurofibrillary tangles, hyperphosphorylation of tau protein and its interaction with alpha-synuclein may promote the death of dopaminergic neurons (Zhang et al. 2018). Importantly, bFGF liposomes ameliorated the phosphorylation of tau via the PI3K/Akt-GSK3β. Moreover, improved delivery of bFGF to the striatum and substantia nigra of rats was reported, showing a significant potential to reverse functional deficits induced by 6-OHDA. Definitely, this strategy could contribute to developing new therapeutic targets to treat PD (Yang et al. 2016a).

The above examples illustrate that liposomal-based strategies for PD have been mainly focused on NF encapsulation, offering novel formulations with enhanced properties. In addition, the combinatorial approach based on the administration of liposomes combined with FUS proved effective to guide the liposomes through the BBB, suggesting an enormous potential to deliver drugs to the brain at targeted locations.

7.6.3 Solid Lipids NPs for PD Treatment

Solid lipid NPs (SLN) (Fig. 7.2c) have been proposed as alternative nanocarriers to overcome the limitations of the existing nanosystems. SLN provide a suitable release profile and excellent stability (Cacciatore et al. 2016). In addition, the use of biocompatible/biodegradable lipids allows the design of noncytotoxic and well-tolerated SLN (Nair et al. 2011; Tapeinos et al. 2017). Unfortunately, SLN have a reduced drug loading capacity, especially for hydrophilic drug. For this reason, SLN would only be proper to encapsulate hydrophobic drugs or hydrophilic drugs effective at very low doses.

7.6.3.1 Neurotrophic Factor Therapies

In this field, chitosan (CTS)-coated nanostructured lipid carriers (Fig. 7.2c) loaded with GDNF were designed by Hernando et al (2018). An interesting point of this study was the strategy used to cross the BBB. To this end, NPs were conjugated with a cell-penetrating peptide named transactivator of transcription (TAT) and intranasally administered in a mouse PD model (Qin et al. 2011; Kanazawa et al. 2013; Hernando et al. 2018). Enhanced locomotor activity was reported together with a significant TH+ neuron recovery. Notably, the number of activated microglia was significantly decreased in both striatum and substantia nigra, while the free GDNF administration did not decrease the level of activated microglia (Hernando et al. 2018; Torres-Ortega et al. 2019). Hence, this is a good example that demonstrates that SLN could be an effective alternative to encapsulate GDNF for PD therapy.

7.6.3.2 Antioxidant Therapies

Previous studies have highlighted the neuroprotective role of curcuma longa to treat PD (Cui et al. 2016; Khatri and Juvekar 2016). This compound possesses a variety of pharmacological applications, including antioxidant, antiinflammatory, antiamyloid, and antiapoptotic functions, which suggests a possible neuroprotective potential. However, the administration of this compound is very limited due to its hydrophobic nature and its consequent low solubility and low oral bioavailability. As a means to overcome this obstacle, Kundu et al. designed curcumin-loaded lipid NPs (Kundu et al. 2016). With this aim in mind, higher bioavailability and biodistribution were achieved by its combination with piperine and its encapsulation into lipid NPs. The NPs effects and the distribution to the brain after oral administration were evaluated in a rotenone-induced PD model. Importantly, curcumin-NPs produced a higher density of TH+ neurons in the *substantia nigra* and an improved motor function. In addition, in vitro inhibition of α-synuclein aggregation into oligomers and fibrils showed the highest inhibitory effect for dual drug-loaded NPs compared to curcumin and piperine (native or nanoformulations).

Overall, these studies demonstrate that solid lipid NPs are a delivery system with high therapeutic efficacy in PD animal models, encapsulating NFs and other molecules such as curcumin.

7.6.4 Inorganic NPs for PD Treatment

Inorganic NPs (Fig. 7.2d) are another class of nanomaterials that have provided significant examples for PD treatment due to their magnetic, mechanical, and chemical properties (Kim and Hyeon 2014).

7.6.4.1 Antioxidant Therapies

As mentioned above, many researchers have investigated the efficacy of antioxidant-based therapies for PD. With this in mind, Umarao et al. tested the antioxidant ability of a dual therapy based on local administration of superparamagnetic iron oxide NPs (Fig. 7.2d) and exposure to magnetic fields (Umarao et al. 2016). In this study, iron NPs showed great antioxidant potential due to their ability for free radical scavenging (Pal et al. 2013). At the same time, the application of an electromagnetic field meant an increase in the antioxidant effect of iron NPs. In vivo tests verified the derived effects from their local administration, finding an enhanced survival of dopaminergic neurons in the striatum, a lower percentage of the volume lesioned, and an improvement in mitochondrial dysfunction. These results support their efficacy to modulate the oxidative mechanisms involved in dopaminergic neuronal death, which makes them promising candidates for PD treatment (Umarao et al. 2016).

Cerium NPs (CeO2NPs) (Fig. 7.2d) are powerful nanomaterials investigated for PD treatment that can counter oxidative stress, act as scavengers of reactive species oxygen (ROS) (Korsvik et al. 2007; Heckert et al. 2008), and undertake enzyme mimetic activities (Heckert et al. 2008). The intraperitoneal administration of CeO2NPs in a PD animal model improved behavioral and neurochemical impairments, producing an increase in the striatal dopamine levels, a decrease in the oxidative stress markers, and an improvement in striatal caspase 3 activity (Hegazy et al. 2017). In another study, Khausik et al described a molecular interaction between CeO2 NPs and α-synuclein at the active sites using computational biology approaches, suggesting a possible potential inhibition of α-synuclein. However, this interaction should be confirmed in in vivo experiments (Kaushik et al. 2018).

Collectively, these studies highlight the exciting potential of inorganic NPs to alleviate the oxidative stress and ROS associated with PD, providing an alternative approach to treat this neurodegenerative disorder. Due to the multifactorial etiology of PD, antioxidant-based NPs may not constitute an approach with enough power to completely restore the nigrostriatal lesion, but they could be a complementary strategy to strengthen other therapeutic options.

7.6.5 Hybrid Nanosystems for PD Treatment

Considering the small size, large specific surface and superparamagnetic properties of hybrid nanosystems (Fig. 7.2e), the encapsulation of drugs into hybrid NPs could be an attractive approach for PD treatment (Vivero-Escoto and Huang 2011).

7.6.5.1 Neurotrophic Factor Therapies

In an alternative strategy, other researchers have developed hybrid nanosystems for DNA plasmid delivery to the brain. For instance, gold NPs (Fig. 7.2e) loading pDNA-nerve growth factor (NGF) and modified by CTS have shown beneficial neuroprotective results in a mouse PD model. The intraperitoneal administration of NPs interfered in the expression of α-synuclein and, in consequence, a significant recovery of the neuronal density in the nigrostriatal pathway was observed (Hu et al. 2018). In this regard, it is worth mentioning that the inhibition of α-synuclein expression could be an important strategy to halt PD progression.

7.6.5.2 Antioxidant Therapies

The design of iron chelators (Fig. 7.2e) as possible treatment for PD has also attracted significant attention. In this regard, Wang et al. designed non-Fe Hemin (NFH) therapeutic NPs to fight abnormal iron accumulation in PD. With this aim, NPs were protected by zwitterionic poly (2-methacryloyloxyethyl phosphorylcholine) and functionalized with a HIV-1 trans-activating transcriptor (TAT) to enhance BBB permeability and to enable the Fe chelation in the brain. NPs were intravenously injected and showed high affinity for Fe ions, prolonged in vivo lifetime, and deferred saturation and ability to reverse functional deficits in parkinsonian mice (Wang et al. 2017).

7.6.5.3 Anti α-Synuclein Therapy

α-synuclein overexpression could be involved not only in the etiopathogenesis but also in disease progression, triggering neuronal death (Thakur et al. 2017; Ganguly et al. 2018). As an example of an attempt to halt α-synuclein overexpression using nanotechnology, Niu et al. developed magnetic Fe3O4 NPs (Fig. 7.2e) coated with oleic acid molecules (Niu et al. 2017). To this end, N-isopropylacrylamide derivative was firstly photoimmobilized onto the oleic acid molecules, and short hairpin RNA (shRNA) plasmid to interfere with α-synuclein was absorbed. Then, NGF was also absorbed in an N-isopropylacrylamide derivative. This last step sought to promote the neuronal uptake of particles via NGF receptor-mediated endocytosis. The presence of NPs in the substantia nigra after its intraperitoneal administration was

confirmed by Prussian blue staining (Niu et al. 2017). Magnetic NPs loaded with α-synuclein RNAi plasmid and NGF halted the overexpression of α-synuclein, which was accompanied by the increase of TH expression (Niu et al. 2017). This technology is very promising, as it suggests that it may be possible to regulate the overexpression of α-synuclein in PD patients.

To sum up, hybrid nanosystems for PD treatment are still at a premature stage and further studies are required to demonstrate their efficacy. Nevertheless, the last studies in the field supported the hypothesis that hybrid NPs have a significant ability to cross the BBB and to deliver therapeutics in a controlled manner, suggesting a promising targeting therapy for PD.

7.6.6 Quantum Dots for PD Treatment

Carbon quantum dots (CQD) (Fig. 7.2f) are carbon nanostructures whose photoluminescence (PL) properties, physical and chemical stability, and low toxicity, are being exploited for their application in the field of nanobiotechnology (Molaei 2019).

7.6.6.1 Anti α-Synuclein Aggregation Therapy

Recently, the potential of graphene quantum dots (GQDs) to inhibit α-synuclein fibrillization and disaggregate fibrils was investigated. In this study, GQDs were described as powerful drugs, able to bind α-synuclein fibrils, inhibit their fibrillization, and with potential to achieve their disaggregation (Fig. 7.3b). The neuroprotective effects were investigated in vivo, demonstrating successful penetration of GQDs in the BBB, a reduction in the loss of dopaminergic neurons by α-synuclein preformed fibrils, and an improvement of Lewy neurite pathology and behavioral deficits (Kim et al. 2018). Of note, this work supports the relevance of this technology to fight against one of the most important mechanisms described so far in the progression of PD, being a very promising strategy. However, some more extensive investigations in clinically relevant animal models (i.e., nonhuman primates) are still necessary to assess clinical translatability. In the future, it would be also interesting to study if it is possible to target GQDs to the striatum when administered by less invasive routes, which would increase the likelihood of transferring this strategy to the clinic.

7.6.7 Exosomes for PD Treatment

The intrinsic ability of exosomes (Fig. 7.2g) to cross biological barriers has emerged as a form of drug delivery for the treatment of several diseases. Specifically, various researchers have recently studied the potential of these natural nanovesicles as drug

delivery carriers in the field of PD. For this purpose, Haney and co-workers loaded the antioxidant molecule catalase into exosomes (Fig. 7.2g). These exosomes were intranasally administered into parkinsonian mice, and proved to be a less invasive way to achieve significant neuroprotective effects in an in vivo model of PD (Haney et al. 2015). Exosomal strategies have also been applied to confront the α-synuclein aggregation responsible for the progression of PD pathology. In this sense, Cooper et al. systemically administered modified exosomes expressing Ravies virus glycoprotein loaded with α -synuclein small interfering RNA (siRNA) (Fig. 7.2g) in transgenic mice. As a result, decreased α -synuclein mRNA and protein levels throughout the brain were reported 7 days after injection, suggesting a reduction in intraneuronal protein aggregates, including in the nigral dopaminergic neurons (Wood et al. 2014). In general, these examples highlight the tremendous potential of exosomes to deliver molecules with important therapeutic activity to the brain, due to their intrinsic capacity to cross the BBB.

7.7 Concluding Remarks

During the last few years, the area of PD research has experienced remarkable advances. However, the current state of diagnostic and therapeutic approaches for PD shows the need for novel strategies to improve PD management. This chapter has highlighted the enormous potential of nanotechnology to develop better diagnostic imaging agents, specific biomarkers and enhanced targeted therapies for PD. Although the tests described above are the most remarkable diagnostic tools available so far, their specificity and sensibility are still not sufficient, and the diagnostic accuracy depends on disease stage. Their accuracy improves as the stage/severity of the disease increases. Hence, the recognition of minimally invasive and disease-specific diagnostic biomarkers is required, especially at prodromal or early stages of the disease. Reliable diagnostic biomarkers will not only help us to improve the differential diagnosis, but will also guide our selection of patients for clinical trials involving neuroprotective therapies. In addition, it would be of interest to develop progression biomarkers to monitor changes related to the disease or the neuroprotective effects of a treatment. To date, biochemical markers have not been demonstrated to be a consistent and confident diagnostic test. Nevertheless, nanohybrid technologies have provided significant evidence of their usefulness, supported by several approaches such as antibody-based diagnosis, gold nanorods and oligomers-based diagnosis. Going further, natural nanosized vesicles have been proposed as a promising alternative diagnostic tool owing to their potential ability to reflect the pathophysiological status of PD patients. In this regard, we should further investigate the potential of these approaches to identify specific biomarkers for PD diagnosis.

Likewise, the application of NPs as MRI agents has only been explored in non-specific molecules for PD targeting general amyloid structures, and further studies focused on the development of nanosystems based on antibodies against the

presynaptic dopamine transporter or against the oligomeric α-synuclein are required. This fact is of great relevance for clinical PD management, since the availability of PET equipment is limited, while MRI is widely available in all hospital imaging services.

Regarding PD treatment, in this chapter, we described specific examples of nanotechnology to improve the stability, delivery, and controlled release of NFs, revealing promising results in preclinical studies. These formulations could solve the problem of lack of efficacy found in previous clinical trials and would enable potentially curative approaches to be developed. Moreover, the great variety of routes of administration (intravenous, intraperitoneal, oral, and intranasal), combined with FUS technology, opens the door to the development of less invasive strategies. Other noteworthy approaches are based on nanomedicines that interfere with α-synuclein aggregation. Recently, GQDs have demonstrated an enormous potential to inhibit α-synuclein fibrillization and disaggregate fibrils, suggesting a promising future.

Thus far, the great majority of nanomedicines have only been tested in models that use toxins to produce a PD-like phenotype in small animals. These models do not fully reproduce the pathological hallmarks and symptoms that occur in humans, such as α-synuclein aggregation and Lewy body formation. Therefore, future work using other types of animal models, such as α-synuclein-based animal models or larger clinically relevant animal models (i.e., nonhuman primates), could help to ratify the effects found in these studies and to transfer their findings to clinical trials.

Additionally, the cooperation among researches, clinicians, and the industry is the key to maximizing the translational potential of nanobiotechnology. A culture of collaboration is needed that allows us to develop more specific strategies, reproducible methodologies with feasible scale-up synthesis, and enhanced data transparency and data sharing.

Thus, despite the current limitations, this particular scenario is highly promising as far as the advancement in nanomedicine research against PD is concerned. New frontiers are being opened that in future may allow a comprehensive approach to the disease through the identification of specific biomarkers, noninvasive procedures, and controlled delivery of drugs to the brain.

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Chapter 8
Selenium Nanoparticles as Therapeutic Agents in Neurodegenerative Diseases

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Abstract The Huntington's disease, amyotrophic lateral sclerosis, Alzheimer's disease, and Parkinson's disease are major neurodegenerative diseases, which cause severe damage to neuron and affect the neurodegeneration function. The nanoparticles such as gold, copper and copper oxide, selenium, zirconium oxide, and silver nanoparticles are highly used in anticancer activities against lung, liver, breast, skin, and colon cancer, etc., and drug delivery systems. The nanoparticles are major drug carriers for delivering very sensitive and highly valuable drug to complicated diseases. Selenium is the important micronutrient of our body and selenium nanoparticles for the biomedical applications are very useful one for the biomedical community. Delivering the drugs across the blood–brain barrier is the complicated process and it needs very sensitive and nanosized particles for the drug delivery. In this context, the selenium nanoparticles play an important role in the application for neurodegenerative diseases. In this chapter, we explain the green synthesis of selenium nanoparticles using eco-friendly methods, their characterization using various analytical techniques and applications in the diagnosis and treatment of diverse diseases.

Keywords Alzheimer's disease · Selenium nanoparticles · Treatment · Diagnosis · Neuron

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8.1 Introduction

Nanomaterials have exclusive physicochemical properties such as nano size, large surface area, and high reactivity. Research has emphasized that with suitable surface modifications, nanoparticles (NPs) can be used as tools for drug delivery, imaging, and biomedical applications (Yang et al. 2018) (Fig. 8.1). Selenium (Se) is an essential mineral with a high nutritional value for the majority of physiological interactions in our body. It plays an essential role in cellular redox regulation, neurodegenerative disorders, Alzheimer's disease, detoxification, immune-system protection, and oxidative stress (Khurana et al. 2019). The conversion of dissolved Se oxyanions to elemental Se(o) by microorganisms is significant (Yazhiniprabha and Vaseeharan 2019) for the remediation of selenium contamination and the biogeochemical cycle of this element (Zhang et al. 2019). Moreover, Se (o) is gaining importance in medical applications and biological activities, low toxicity, and enhanced photoelectrical properties.

Biological means of nanoparticle synthesis by plants, bacteria and fungi are cheaper to produce, and also doesn't produce any toxic wastes as by-product. Our body intake of selenium is provided from food or water as selenite, selenocysteine, selenomethionine, and is carried by different human selenoproteins in the body. Humans with selenium deficiency are amenable to many health problems in a way that abnormal selenoprotein function or selenium deficiency could lead to various neurological thyroid muscle and many other physiological disorders. So, the need for selenium starts from an early age and continues until old age.

Selenoproteins are proteins containing selenium as amino corrosive selenocysteine. Selenoproteins are, for the most part, communicated in the human cerebrum and in all likelihood associated with cell reinforcement forms. Selenium can also have a direct antioxidant effect on the brain and neurons. Selenium forms several allotropes (red, black, and gray) that interconvert with temperature changes. It has

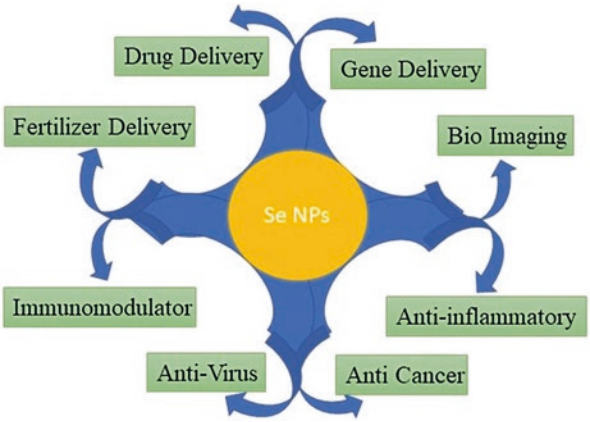

Fig. 8.1 Application of Se nanoparticles

two different physiological features. Concentration-dependent selenium can exert either its therapeutic or toxic effects. High dosages of selenium advance the multiplication of malignant growth cells and have neurotoxic impacts, although low and middle portions restrain disease cell expansion, and affect neurological illnesses.

The outstanding metal-rich nanoparticles, for example, TiO_2 ZnO iron oxide SiO_2 silver (Ag) and gold (Au) have, for the most part, oxidative impact in the cerebrum. The selenium-rich nanoparticles with antioxidant properties such as red selenium and sodium, selenite are used in the treatment of brain diseases, and they size-dependently improve cognitive functions. Cancer prevention agent and mitigating impacts of selenium were seen in vitro utilizing cell lines and in vivo in creature models.

Homocysteine is known to increase the danger of building up Alzheimer's ailment and is found in raised levels in patients with AD. Choline plays out a compound change, changing over the destructive homocysteine into the accommodating concoction methionine. (Velazquez et al. 2019). The joining of these two fields is especially useful for the combination of diagnostics and treatment, commonly termed theranostics (Vissers et al. 2019). Here, we review the current status of using nanomedicine along with stem cell therapy to diagnose, track progression, and treat neurodegenerative diseases. With this motivation in mind, this chapter is focused on selenium nanoparticles synthesized by eco-friendly method and used in Alzheimer's disease, neurodegenerative disorders, and oxidative stress.

8.2 Green Synthesis, Characterization and Biomedical Applications of Selenium Nanoparticles

Selenium nanoparticles are synthesized using different methods such as physical, chemical, and biological. In the biological method, researchers are using fungi bacteria and plants for the preparation of selenium nanoparticles. Figure 8.2 shows the schematic representation of the green synthesis of selenium nanoparticles, and its various characterization techniques with different applications.

8.2.1 Fungal-Mediated Synthesis of SeNPs

Sarkar et al. (2011) synthesized selenium nanoparticles using *Alternaria alternata.* They used fungal extract for the bioreduction of sodium selenite to produce selenium nanoparticles. The change in color to dark red signifies the development of Nano-α-selenium. Dynamic light scattering experiments, atomic force microscopy, scanning and transmission electron microscopic images explained the formation of monodisperse, spherical α-selenium nanoparticles in the range of 30–150 nm. X-ray diffraction spectrum of the nano-selenium exhibited a broad peak at a 2θ angle of

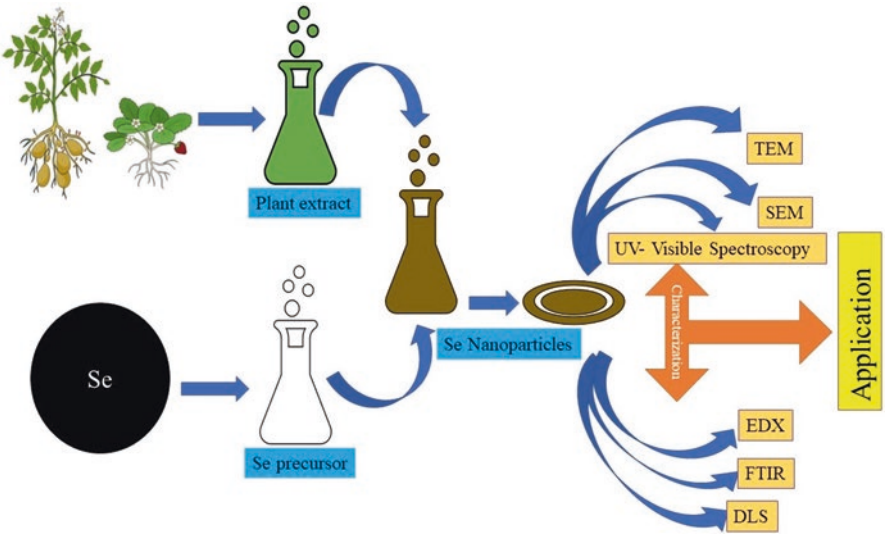

Fig. 8.2 Schematic diagram of biosynthesized Se nanoparticles and characterization

15–35 signifying its amorphous nature. Energy dispersive X-ray study revealed the presence of selenium in the nanoparticles. The size of the selenium nanoparticles analyzed was between 0.6 nm and 6.0 μm. The shape of selenium nanoparticles was monodispersed spherical in the range of 30–150 nm.

Zare et al. (2013) also synthesized selenium nanoparticles by using fungus; they characterized the selenium nanoparticle by UV-Vis spectroscopy and energy dispersive x-ray spectrum. The size of the nanoparticles was 47 nm with a spherical shape. UV-Vis spectroscopy and energy dispersive X-ray spectrum studies were carried out to confirm Se NPs formation within 60 min. Dynamic light scattering and scanning electron microscopic methods were also used to characterize both the size and shape of the nanoparticles (Zare et al. 2013).

8.2.2 Bacterial-Assisted Synthesis of SeNPs

Fesharaki et al. (2010) synthesized selenium nanoparticles using strains of *Klebsiella pneumoniae*. This bacterium was tested for its ability to synthesize elemental selenium nanoparticles from selenium chloride. This method used broth of *Klebsiella pneumoniae* culture and was subjected to sterilization at 121 °C and 17 psi for 20 min. Released selenium nanoparticles ranged from 100 to 550 nm with the average size of 245 nm (Fesharaki et al. 2010).

Srivastava and Mukhopadhyay (2013) reported synthesis of selenium nanoparticles using bacteria *Zooglea ramigera.* They characterized the nanoparticles using DLS, SEM, EDAX, SAED, and XRD. The size of the nanoparticles was 78 nm and

210 nm. A transmission electron microscope characterized the synthesized Se nanoparticles at an accelerating voltage of 200 kV dynamic light scattering particle size analyzer and scanning electron microscopy with an energy dispersive X-ray analysis for the size and morphology and elemental analysis of the product. XRD analysis was carried out to examine the crystalline phase of synthesized nanoparticles.

Srivastava et al. (2014) synthesized selenium nanoparticles using *Halococcus salifodinae* BK 18 and characterized nanoparticles using X-ray diffraction, selected area electron diffraction and transmission electron microscopy. The rod-shaped nanoparticles of 28 nm size were formed. The TEM images were obtained using Philips TEM operated at an accelerating voltage of 190 keV. The elemental composition of the nanoparticles was determined by disruptive energy analysis of X-ray, scanning electron microscope equipped with EDX operating at 20 keV.

Rajasree (2015) also synthesized selenium nanoparticles using three species of non-pathogenic, eco-friendly, and readily available lactic acid bacteria (LAB): *Lactobacillus acidophilus*, *Lactobacillus plantarum,* and *Lactobacillus rhamnosus*. Lactic acid bacteria can reduce selenium ions to elemental selenium nanoparticles and deposit them in intracellular spaces. The reduction of selenium ions in metal nanoparticles was investigated virtually by tracing the solution color, which changed from red after 48 h. The shape of the selenium nanoparticles was spherical as visualized by TEM. The size ranged from 20 to 150 nm by UV spectrum. Sasidharan and Balakrishnaraja (2014) synthesized selenium nanoparticles by various microorganisms. They characterized the nanoparticles by TEM and UV-Visible spectroscopy. The size of the nanoparticles was 245 nm with spherical shape.

Shakibaie et al. (2015) synthesized in another study in which Selenium nanoparticles were synthesized from *Bacillus* species *Msh-1* and characterized by the help of TEM, X-ray, and EDX. The size of spherical nanoparticles was 120–140 nm. Energy-dispersive X-ray spectroscopy demonstrated that the purified NPs consisted of only Se.

In a study, Srivastava and Kowshik (2016) used moderately halophilic bacterium *Idiamarina* sp. PR58-8 and sodium selenate as a precursor. The nanoparticles were characterized by XRD which exhibited the characteristic Bragg's peak of hexagonal selenium with the crystalline domain. Morphological characterization by TEM showed spherical nanoparticles of size 150–350 nm. The selenium nanoparticles induced caspase-dependent apoptosis in HeLa cell lines as exhibited by ROS assay, apoptotic index assay, and Western blot analysis. These results suggest the application of SNP's synthesized by *Idiamarina* sp. PR58-8 as potential anti-neoplastic agents (Srivastava and Kowshik 2016). Kora and Rastogi (2016a, b) synthesized selenium nanoparticles by *Pseudomonas aeruginosa* ATTC27853. The generation of selenium nanoparticles was confirmed from the appearance of red color in culture broth and broad absorption peaks in the UV-vis-spectrophotometer. The shape of selenium nanoparticles was spherical, polydispersed in the range from 47 to 165 nm. The average size of the nanoparticles was 95.9 nm. They concluded that additional products such as seleno-amino acids and volatile alkyl selenite might have been formed during selenite transformation (Kora et al. 2016). The authors synthesized selenium nanoparticles from *Escherichia coli* ATTCC 35218. The

shape of selenium nanoparticles was spherical polydisperse in the range from 100 to 183 nm. The average size of the nanoparticles was 155 nm (Kora and Rastogi 2016a, b). Fernández-Llamosas et al. (2016) synthesized selenium nanoparticles using *Azoarcus* sp. CIB. Electron microscopy and X-ray spectroscopy demonstrated the reduction of selenite to spherical electron-dense selenium nanoparticles. The average size of SeNPs was 123 ± 35 nm. Since *Azoarcus* can debase both vigorously and anaerobically dangerous sweet-smelling mixes of extraordinary ecological concern, it turns into an appropriate contender for a progressively maintainable agrarian practice and bioremediation strategies (Fernández-Llamosas et al. 2016). They reported the synthesis of selenite nanoparticles using *Stenotrophomonas maltophilia* Se ITE02. The particles were characterized by using SEM, TEM, and EDX. The size of spherical/ovoid nanoparticles was 160–250 nm (Lampis et al. 2017).

Khoei et al. (2017) synthesized selenite nanoparticles by *Burkholderia fungorum* which were characterized by using SEM, TEM, and DLS. The size of the nanoparticles was 170–200 nm.

Kim et al. (2016) synthesized europium selenide (EuSe) nanoparticles by in vivo method using recombinant *Escherichia coli* cells expressing heavy metal-binding proteins phytochelatin synthase metallothionein. The formation of EuSe nanoparticles was confirmed by using UV-vis spectroscopy, X-ray diffraction, and transmission electron microscopy. The synthesized EuSe nanoparticles exhibited high fluorescence intensities as well as strong magnetic properties. The anticancer effect of EuSe nanoparticles against cancer cell lines was investigated. The size and morphology of biogenic EuSe nanoparticles were characterized by TEM at an accelerating voltage of 300 kV with 0.2 nm point resolution. After a 6-h exposure to EuSe nanoparticles, a significant emission peak corresponding to the EuSe appeared in the cell suspension; however, no emission peak was observed in the untreated cell pellet suspension. The XRD pattern showed peaks. The particle sizes of the EuSe nanoparticles from several concentrations of precursors (1 mM, 2 mM, 3 mM and 5 mM) obtained by the FWHM of the peaks were as obtained to be 1.74 ± 0.18 nm, 1.93 ± 0.28 nm, 2.14 ± 0.27 nm, and 2.58 ± 0.29 nm in diameter, respectively (Kim et al. 2016).

Shirsat et al. (2016) synthesized selenium nanoparticles by using microorganisms and characterized the nanoparticles by using XRD, plane-view of scanning electron microscopy digital photo images. The average size of the nanoparticles was 110–150 nm. Yan et al. (2018) synthesized selenium nanoparticles by using carboxylic curdlans. The size of the nanoparticles was 50–90 nm, and the shape of the nanoparticles was spherical. They were characterized by UV-Vis spectrophotometer in the wavelength range of 190–600 nm with an interval of 1.0 nm. The particle size and morphology of the SeNPs were observed using TEM, and EDX determined the elemental composition of the SeNPs. The crystalline size and structural property of the SeNPs were determined by X-ray diffraction. Qi et al. (2019) synthesized selenium nanoparticles by using bacterium *Pantoea agglomerates.* Analysis using X-ray photoelectron spectroscopy, Fourier transform infrared spectroscopy, and X-ray energy dispersive spectroscopy followed by optical characterization using

ultraviolet-visible confirmed that the biosynthesized Cu^2-Se nanospheres were capped by protein. SEM evaluated the morphologies and the dimensions of the nanospheres.

Presentato et al. (2018) synthesized selenium nanoparticles by the aerobic bacterium *Rhodococcus aetherivorans BCP1*. The nanoparticles were characterized by using TEM EDX DLS and zeta potential measurements. Zeta potential measurements were conducted to evaluate the surface potential of Se-nanostructure extracts. The size of the nanoparticles was 50–500 nm, and the shape of the nanoparticles was spherical.

Tareq et al. (2018) synthesized selenium nanoparticles using *Clostridium botulinum*. The selenium nanoparticles were characterized using atomic absorption spectroscopy, Fourier transform infrared spectroscopy X-ray diffraction, scanning electron microscope, and transmission electron microscope. The selenium nanoparticles were synthesized successfully as the nano-crystalline pure hexagonal phase and the size range of 26–41 nm with a spherical shape. In the FTIR spectrum, the sharp absorption peaks arose at 1642 and 3295 cm^{-1}. The biomolecule synthesized selenium nanoparticles produced at nano-size with spherical shape. The average nanoparticles were synthesized at size 35 nm. Xu et al. (2018) synthesized selenium nanoparticles from *Lactobacillus casei ATCC 393*.

8.2.3 Plant-Mediated Synthesis of SeNPs

Ramamurthy et al. (2013) synthesized selenium nanoparticles by using Fenugreek seed extract. UV-vis spectroscopy SEM, FTIR, XRD, and XRF characterized these selenium nanoparticles. The size of the nanoparticles was 50–150 nm. SEM images were taken for the analysis of size and shape of SeNPs with a resolution of 500 nm operated at 10 kV HV mode, and detectors contained secondary electron. Li et al. (2014) synthesized selenium-containing phycocyanin for prevention of beta-cell apoptosis. Human islet amyloid polypeptide (hIAPP) fibril is the principal constituent of amyloid deposits in pancreatic islets of type 2 diabetes. Studies have shown that selenium-containing phycocyanin (Se-PC) inhibited the fibrillation of hIAPP from forming nanoscale particles, which are mainly by interfering with the combination between hIAPP. Small nanoscale oligomers tended to grow into larger nanoparticles, and the size of nanoparticles increased with the incubation time. Se-PC alleviated hIAPP-induced cell apoptosis. The selenium nanoparticles were characterized by atomic force microscopy and TEM.

In another study, Deepa and Ganesan (2015) reported synthesis of selenium nanoparticles by flower extract of *Catharanthus roseus.* The authors characterized the nanoparticles using UV-visible spectroscopy LS, FT-IR, XRD, EDAX, SEM, TEM, and SPR. The size of the nanoparticles was 32.02 and 40.2 nm, and the shape of the nanoparticles was hollow spherical. UV-Vis spectroscopic analysis showed the surface plasmon resonance vibrations at 335 and 325 nm. FT-IR analysis sub-

stantiated the role of esters secondary and tertiary amides derived from the flower of *C. roseus* in the synthesis and stabilization of SeNP.

The extract of *Allium sativum* was used by for synthesis of selenium nanoparticles. Characterization studies revealed the formation of crystalline spherical-shaped SeNPs. The SeNPs–DNA interactions were directly visualized by atomic force microscopy. The present investigation showed harmless, very stable SeNPs complicated component of DNA association, which can be an achievement in DNA focused on chemotherapy. Sowndarya et al. (2017) also used plant for synthesis of selenium nanoparticles. They selected *Clausena dentata* plant leaf extract for synthesis of SeNPs and characterized using FTIR, EDAX, and SEM. The size of the nanoparticles was 46.32–78.88 nm. In UV-vis spectroscopy high absorption spectrum was observed at 240 nm. FT-IR identified the possible band present in the biomolecule responsible for peaks near capping and efficient stabilization of the metal NPs synthesized by leaf extract. In EDAX peaks, around 72.64 to the binding energies of selenium were observed (Sowndarya et al. 2017).

Tamminen et al. synthesized selenium nanoparticles by using *Piper nigrum* seeds; they characterized the nanoparticles using TEM, XRD, DLS, and FTIR. The size of the nanoparticles was 8.85 ± 3.5 nm. In XRD, the diffraction pattern revealed the phase purity and crystallinity of SnO_2NPs. DLS determined the average particle size of the NPs with a polydispersity index (PDI).

8.3 Biomedical Applications of SeNPs Synthesized Using Physical and Chemical Methods

Vekariya et al. (2012) synthesized stable selenium nanoparticles and elucidated their mechanism of action in preventing the growth of mammary tumors. The size of the nanoparticles was 40–90 nm. The nanoparticles were characterized by using UV-Visible spectrophotometer, TEM, and SEM.

Akar et al. (2013) synthesized selenium nanoparticles by the reduction of aqueous sodium selenite solution with freshly prepared glucose solution. This method was capable of producing spherical selenium nanoparticles in a size range of about 150–175 nm under ambient conditions. Moreover, Se/PES and Cu/PES blend membranes were also characterized using scanning electron microscopy (SEM) and permeation tests.

Guisbiers et al. (2017) synthesized selenium nanoparticles by pulsed laser ablation in liquids for inhibition of the *Candida albicans*. Selenoproteins play an essential role in the human body by accomplishing essential biological functions like oxide-reductions antioxidant defense thyroid hormone metabolism and immune response. Those particles have been successfully used to inhibit the formation of *C. albicans* biofilms. Innovative electron microscopy images exposed that selenium nanoparticles easily adhere on the biofilm and then penetrate the pathogen and consequently damage the cell structure by substituting with sulfur; 50% inhibition of

C. albicans biofilm was obtained at only 25 ppm. The two physical parameters that proved to affect the viability of *C. albicans* firmly are the crystallinity and particle size. The bulk selenium pellets used as a target during the synthesis were characterized by Raman spectroscopy (Guisbiers et al. 2017). Hossain et al. (2018) synthesized selenium nanoparticles and used-on cadmium-induced cytotoxicity in PC12 cells via regulating oxidative stress and apoptosis. This study investigated mechanisms of cytoprotecting by selenium (Na2SeO3; Se4+) against cadmium ($CdCl_2$; Cd_2+)-induced cytotoxicity using PC12cells. Also, Se (5, 10, 20 and 40 μM) and Cd (2.5, 5 and 10 μM)-induced cytotoxicity was determined (Hossain et al. 2018). Kaur et al. (2018) synthesized CdSe/ZnS nanoparticles and papain. The interactions among carboxyl-surfaced Cadmium selenide/Zinc sulfide (CdSe/ZnS) core/shell nanoparticles (NPs) with papain were studied by various spectrometric methods. The binding constants for papain-CdSe/ZnS NPs conjugates were determined by analyzing the fluorescence quenching of papain at different temperatures. The size of CdSe/ZnS NPs so obtained was 4.0 ± 1.2 nm from TEM images. Fluorescence analysis was carried out on Shimadzu Spectro fluoro photometer (RF-5301PC) equipped with a thermostat bath. The measurements were made at 25 °C and scanned five times in the range 200–250 nm for far UV-CD and 250–350 nm for near UV-CD studies and finally averaged (Kaur et al. 2018).

Shi et al. (2018) reported synthesis of selenium nanoparticles, and exposed nanoparticles led to malformation in offspring. The resultant CTS-SeNPs existed as monodispersed spherical particles in aqueous solution. These CTS–SeNPs were characterized by transmission electron microscopy and Nano Sight NS300 for particle size distribution. EDX characterized the elemental composition of the SeNP under TEM. ICP-MS determined total Se concentration of this CTS-SeNP stock. The synthesized CTS-SeNPs were spherical (Fig. 8.2) and had a mean particle diameter of 68.6 nm (SD = 9.5) as defined under TEM and 129 nm (SD =40 nm) as determined by Nano sight (Shi et al. 2018). Synthesized selenium nanoparticles from electronic waste showed functional morphological characterization. The size of the nanoparticles ranged from 50 to 500 nm, with an average size between 50 and 200 nm. The shape of the nanoparticles was spherical. The analysis of EDAX the SeNPs produced peaks at 1.37 keV, 11.22 keV, and 12.49 keV, which were absorption peak of SeNPs (Eswarapriya and Jegatheesan 2015).

Li et al. (2018) synthesized selenium nanoparticles by using natural polysaccharides. They are characterized by using gel permeation chromatography, gas chromatography, FT-IR, NMR, and AFM. The size of the nanoparticles was 42–92 nm, and the shape of the nanoparticles was spherical. The morphology and size of polysaccharide conjugated selenium nanoparticles can be detected by transmission electron microscopy (TEM). Elemental composition and distribution analysis can be determined by Energy Dispersive X-Ray Spectroscopy (EDX).

Ahluwalia et al. (2016) synthesized selenium nanoparticles as co-catalyst for the enhanced photocatalytic activity of as-prepared ZnS photocatalytic for degradation of methyl orange dye under UV light irradiation. They are characterized by using UV–visible spectroscopy DLS, XRD, SEM-EDX, TEM, and BET. The band edge absorption at 330 nm significantly red-shifted at 375 nm. Cary et al. (2017) synthe-

sized selenium nanoparticles derived from metamorphic rocks of Himalaya in Punjab. Coal also contains the necessary amount of selenium, and it also provides antioxidant activity.

8.4 Neurodegenerative Disorders

The "Neurodegenerative Disorders" (NDs) speak to conditions which are irregular and familial and are additionally described by the continuous loss of sharpness in points of view of memory and talent. NDs share a few similitudes in subcellular and atomic level, for example, synaptic variations from the normal neuronal misfortune and event of cerebral stores which are brought about by the total of misfolded proteins. The most common NDs are Alzheimer's disease (AD), Parkinson's disease (PD), epilepsy, and Huntington's disease (HD). The superior medicines for the neurodegenerative condition are regularly inaccessible because of the reduced availability of therapeutic medications. Also, the blood–brain barrier (BBB) adequately obstructs the exchange of cell particles and massive atoms, that is, drugs across the brain. The most critical challenge in the treatment of neurodegenerative diseases is the development of targeted drug delivery system.

8.4.1 Alzheimer's Disease

Alzheimer's Disease (AD) is the world's most regular type of dementia. Frequency of AD is as yet expanding on the planet, and the advancement of new techniques is required for the treatment of AD. In spite of the high number of studies, AD has not been explained at this point. There are numerous hypotheses to clarify the reason for Alzheimer's.The collection of amyloid antecedent protein shaping amyloid-β (Aβ) plaques involves in (1) hyperphosphorylation of tau protein, (2) oxidative pressure, (3) modifications in cholinergic neurotransmission, (4) ecological contamination, (5) hereditary elements including changes of amyloid forerunner protein (APP) and presenilin (PSEN) qualities, (6) resistant framework brokenness, and so forth.

Both reactive oxygen species (ROS) and reactive nitrogen species (RNS) are considered to play essential roles in the induction of AD. Also, Aβ section 25–35 (Aβ25–35) initiates enactment of inducible nitric oxide (NO) synthase and protein oxidation in hippocampal fibroblasts got from AD patients, and they seem, by all accounts, to be in charge of ROS and RNS-instigated hippocampal harm. Also, the ROS and RNS initiate other injury processes such as neuroinflammation and protein misfolding in the AD brain. A few reports demonstrate that sulfur molecules are likewise associated with the oxidation procedure. For instance, the oxidative pressure-instigating properties of Aβ-peptide (1–42) are cancelled by substitution of the sulfur atom of methionine at position 35 with a methylene group.

8.5 Oxidative Stress

The two major types of free radical species are reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS are synthetically sensitive species containing oxygen. Models incorporate peroxides superoxide hydroxyl radical and singlet oxygen. They are delivered during the physiological procedures. For instance, immersed microscopic organisms and infections in the neutrophils are executed by the ROS. The two ROS and RNS are given in the cerebrum because of presentation of natural factors, for example, electromagnetic radiation and mechanical component contamination. Harmful nanoparticles, for instance, aluminum or manganese oxide nanoparticles from air contamination can prompt overproduction of ROS and oxidative damage in mind. They can quicken the movement of neurodegenerative ailments, for example, AD amyloid parallel sclerosis, and Parkinson's sickness. On the off-chance that cancer prevention agents do not control the measure of ROS, they will make noteworthy reversible or irreversible harm a broad scope of organic particles including nucleic acids lipids and proteins or any adjacent atom causing a course of chain responses. There is considerable interest in the ROS-induced reactions and their relationship in the physiology and pathology of NDs.

8.5.1 Role of Selenium on Oxidative Stress

Selenium is a fundamental component in our body. One of the necessary selenium-subordinate detoxifying procedures is related to the action of GSH-Px catalyst. GSH-Px protein contains a selenocysteine (Se-Cys) moiety in its dynamic site. GSH-Px catalase and SOD compounds have synergistic capacities in the evacuation of H_2O_2 and natural peroxides. GSH-Px catalyzes a reaction wherein two reduced monomeric glutathione (GSH) react with H_2O_2 and structure oxidized glutathione (GS-SG) and H_2O. GSH contains thiol bunches in its structure. GS-SG is diminished back to its thiol structure (GSH) by the glutathione reductase catalyst. Thioredoxins are small peptides in the cytosol and mitochondria assumes an essential job in keeping up a diminished domain in the cells through thiol-disulfide trade response and shields cells and tissues from oxidative pressure. Decrease of thioredoxin is catalyzed by thioredoxin reductase-1 (Trx1), and most radicals, for example, hydrogen peroxide (H_2O_2) and nitric oxide (NO). Since cancer prevention agents can hinder Aβ accumulation pathways, new treatments have been produced for AD. As it was referenced above, thioredoxin (Trx) and glutathione (GSH) are the two noteworthy frameworks which assume a significant job in the upkeep of cell redox homeostasis. It was accounted for that the hindrance of GSH blend increased Aβ-induced cell death and intracellular Aβ accumulation. Diminished degrees of selenium GSH and GSH-Px were seen in model creatures and AD patients (Zhang et al. 2013). The relationship between the loss of subjective capacity and plasma selenium levels was likewise seen in patients with AD. Along these lines, the impor-

tance of selenium and GSH redox framework in patients with AD shows their significance in redox regulation. Diminished selenium substance in plasma and hair were accounted for in the patients with AD, and a negative relationship was discovered between the selenium content in plasma and rate of AD. Additionally, selenium medicines diminished the degrees of oxidative pressure and Aβ arrangement in the cerebrum of exploratory creature models for AD. Positive relationships among plasma and hair selenium lack and psychological decay were accounted for in patients with AD. Recently selenium is attributed with tau Aβ42 GSH lipid peroxidation and a cancer prevention agent catalyst from the blood tests of AD patients in South India. No relationships were accounted for between the degrees of selenium all out tau and Aβ42.On the contrary, decreased GSH-Px enzyme activity and reduced levels of selenium were reported in erythrocytes of patients with dementia and AD. In addition to the oxidant nanoparticles, there are nanoparticles with cell reinforcement properties, for example, selenium. Mitochondria are the primary source of ROS such as superoxide and hydroxyl radicals in mammalian cells during normal energy metabolisms. Selenium consumed in foods and supplements exists in several forms including selenomethionine, selenocysteine, selenite and selenide. The reduced form of sodium selenite is selenide, and it is produced in cytosol by catalytic effects of the GSH and superoxide radicals. Then, the selenide is transformed to methylselenol, and it is further converted into methylated metabolites. Also, the selenide in cytosol serves as a source for selenoproteins selenium sugar and elemental selenium. Redox administrative and ROS rummaging exercises of the selenium nanoparticles with various sizes have been accounted for. ROS rummaging impacts of red selenium nanoparticles with little (5–15 nm) medium (20–60 nm) and enormous (80–200 nm) sizes were examined in an in vitro model, and the most astounding ROS searching impact was seen on account of little sized red selenium nanoparticles. Red natural selenium nanoparticles with 20–60 nm sizes are delivered from sodium selenite by the initiated GSH redox framework, and they instigated the multiplication rate of human hepatic disease cells in vitro. They induced an increased activity of GSH-Px and Trx1 enzymes compared to sodium selenite.

On the contrary, revealed that GSH-Px and Trx1 exercises were influenced neither in human hepatoma HepG2 cells nor in the mice liver by the organization of selenium-rich nanoparticles in a size scope of 5–200 nm. Also, the size-dependent protective effect of selenium nanoparticles against ROS production and DNA damage has been found.

8.5.2 Role of Selenium Nanoparticles in NDs

Nanoparticles are having a lot of advantages in the neurodegenerative diseases as shown in Fig. 8.3. Trace element selenium as an essential nutrient has a significant health effect in human biology. Accumulating evidence indicates the important role of selenium in redox regulation because there are considerable data on the positive correlation between increased cognitive decline and decreased selenium level.

The redox cycles of selenium (II) sodium selenite (VI) and sodium selenite (IV) structures are believed to be the most significant instrument connected to the biological systems in the brain by the inhibition of ROS. Various structures and sizes of selenium can impact its presentation. The basic selenium nanoparticles have a size somewhere in the range of 20 and 500 nm, and they are additionally alluded to as red or essential selenium. The size of essential red selenium shaped was reliant on the measure of protein in the redox framework. For instance, it was accounted for in two late investigations that selenium nanoparticles somewhere in the range of 20 and 60 nm in size have comparable bioavailability to sodium selenite (Zhang et al. 2013). In the course of the most recent decade, other selenium-containing cancer prevention agent nanoparticles pulled in high interest for human neurobiology because of their inhibitory impacts on oxidative pressure. The delayed consequences includes that of sialic destructive adjusted selenium nanoparticles in neuronal cell line (PC12) cytotoxicity, the mitochondrial film depolarization-provoked oxidative weight and apoptosis levels.

Gathering proof shows that there is an immediate connection between the insufficiency of selenium in serum and hair tests and memory deficiency in patients with AD (Kaya et al. 2015). It has been accounted for that selenium treatment may diminish the danger of memory shortages in creature models and AD patients (Demirci et al. 2017). Recent interests are developed around the job of selenium and selenoproteins in neurodegenerative maladies, including AD.

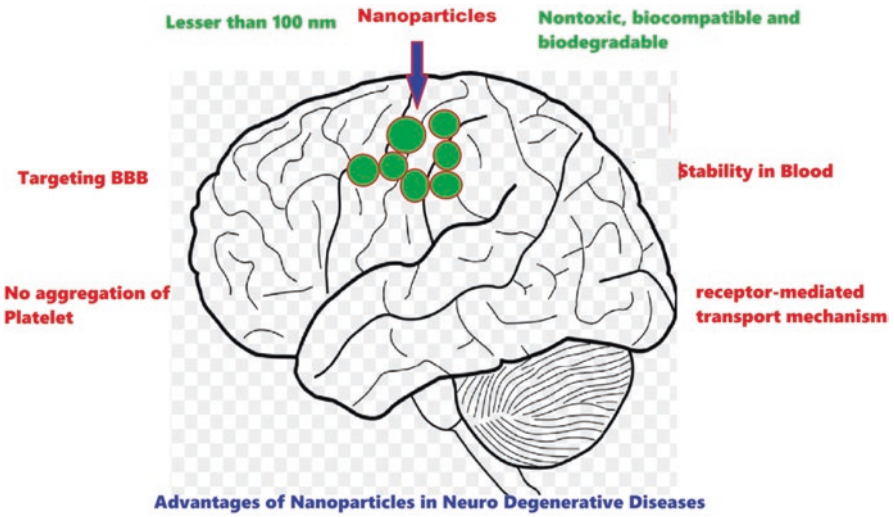

Fig. 8.3 Advantages of NPs in neurodegenerative diseases

8.6 Conclusion

The selenium nanoparticles have shown significant activity in the free radical scavenging, anti-cancerous effect and drug delivery systems. So, in future, it may be used in many biomedical applications due to its nanostructure and its biocompatible nature. There are some recently orchestrated selenoproteins and selenium nanoparticles having amazing physiological properties. Because of the high strength and low fundamental lethality of these particles, they may fill in as options for helpful medications in the treatment of neurological illnesses, including Alzheimer's disease.

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Chapter 9
Role of Supermagnetic Nanoparticles in Alzheimer Disease

Shanmugam Rajeshkumar, Devaraj Ezhilarasan, Napaphol Puyathron, and Thangavelu Lakshmi

Abstract Nanoparticles are the major development of nanotechnology and used in different biomedical fields. The nanoparticles are in different forms such as metal, polymer, and composites. In the metal and metal oxide, nanoparticles are used as anticancer, antimicrobial, and in many neurological diseases. The magnetic nanoparticles play an important role in the diagnosis, especially the imaging techniques (magnetic imaging, magnetic resonance imaging MRI scanning) and many sensors and environmental remediation techniques. Recently, the nanoparticles were used in the neural stem cell detection and neurodegenerative diseases, especially the Alzheimer's disease. We have very limited numbers of research articles in the area of magnetic nanoparticles in neurodegenerative diseases. In this chapter, we have elaborated the synthesis of magnetic nanoparticles using different plant extracts and microorganisms used for magnetic nanoparticles synthesis and characterization of the nanoparticles using different microscopic and spectroscopic techniques and biomedical applications of magnetic nanoparticles. Finally, the magnetic nanoparticles in the Alzheimer's disease have been explained with graphical representations.

Keywords Magentic nanoparticles · Iron oxide · Neurodegenerative · Green synthesis · Biomedical · Alzheimer's disease

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9.1 Introduction

Nanoparticles are particles below 100 nm. Depending on the dimension, it is referred to as the quantum dot (0-D), nanowire, or nanotubes (1-D), nanofilm (2-D) or nanostructure (3-D). It is well known that increasing or decreasing surface area/volume ratio of material can alter their many properties such as mechanical strength, electric polarization, magnetization, thermal and catalytic properties. So, these properties lead them to apply in biomedical fields (Mehta 2017; Rajendran et al. 2018).

Recently, many kinds of metal NPs and metal oxide NPs were available on the biomedical field for treatment and diagnosis. Iron oxide nanoparticles (IONPs) are stable, biocompatible, and magnetic in nature. Hence, their magnetic property is used extensively in the biomedical field as magnetic resonance imaging contrast agents, cancer treatment by magnetic hyperthermia, and cancer theranostics (Tartaj et al. 2003; Collingwood and Telling 2016; Nedyalkova et al. 2017; Mehta 2017; Saratale et al. 2018).

This chapter provides conceptual information on methods of IONPs synthesis, characterization, and its use in Alzheimer disease.

The key question of NPs synthesis is how to control the size, shape, stability, and dispensability of NPs in desired solvents. Currently, we have many studies on IONPs synthesis technique to answer this question. Several review papers categorize these techniques under three groups: physical, chemical, and biological techniques. Figure 9.1 shows the different methods used in magnetic nanoparticles synthesis.

The physical and chemical techniques are the conventional techniques for synthesis of IONPs. The chemical technique includes co-precipitation method, the hydrothermal method, sol-gel method, microemulsion method, thermal decomposition, sonochemical, Microwave-assisted synthesis, electrochemical, and flow injection technique. The physical technique includes pyrolysis method, Laser ablation synthesis in solution (LASiS.) (Salazar-Alvarez et al. 2006; Wu et al. 2008; Kharissova et al. 2013; Ramimoghadam et al. 2014; Kefeni et al. 2017; Abu-Dief and Abdel-Fatah 2018; Arias et al. 2018) (Table 9.1).

Other than these conventional synthesis techniques, biosynthesis method is also used. Biosynthesis is "Green-synthesis of NPs" because of its environmentally friendly approach, simple, low energy consumption, nontoxicity, cost saving, and effective synthesis process. It includes synthesis by microorganisms and biomolecules (Shamaa et al. 2019; Pranati et al. 2019; Aditya et al. 2019). The synthesis by microorganism is carried out by using microbial enzyme, protein, and bioactive molecules. The biomolecules are used as a precursor for being nuclei of NPs or stabilizing/capping agent for NPs (Rajeshkumar et al. 2019; Nagaraja et al. 2019). This method is easier than synthesis by microorganism because it needs no culture of the microorganism, easy to control condition during synthesis, and bio-molecule is found in nature easily such as plant extracts. Therefore, many researchers use plants' extract to produce NPs as given in Table 9.2 (Kharissova et al. 2013; Saratale et al. 2018).

Fig. 9.1 Different methods used in magnetic nanoparticles synthesis

9.2 Biomedical Applications of IONPs

9.2.1 Antibacterial and Antifungal Applications

Many metal and metal oxide nanoparticles in general and IONPs in particular have been confirmed as antibacterial and antifungal agents (Fig. 9.2 and Table 9.3). Due to multiple studies of IONPs aimed to antibacterial and antifungal study. The mechanisms of IONPs include the following:

1. They alter the potential of the microorganism cell membrane by physical blockage against K^+ Chanel. Effect to K^+ inside the cell is accumulated and depolarized their cell membrane (Warren and Payne 2015).
2. They generate relative oxidative stress (ROS). ROS are generated under photo or chemical reaction by inducing IONPs. IONPs can induce generation of ROS at

Table 9.1 Description of each conventional method

Method	Description	Reference
Co-precipitation method	Use aqueous solutions, which contain the mixture of salts of Fe^{2+} and Fe^{3+} ions in the mole ratio 1:2, respectively. And conducted in an alkaline medium	Wu et al. (2008), Kefeni et al. (2017), Abu-Dief and Abdel-Fatah (2018), and Arias et al. (2018)
Hydrothermal method	Oxidation reaction occurs by exposing iron precursors to vapor in a sealed container, under high pressure and temperature conditions	Wu et al. (2008) and Arias et al. (2018)
Sol-gel method	Use reaction between iron alkoxides and its salts for forming gel and precipitate particles in the matrix of gel	Pandey and Mishra (2011) and Arias et al. (2018)
Microemulsion	Use precipitation reaction by interchanging inner phase of microemulsion mixing between two reactants of inner phase	Wu et al. (2008), Malik et al. (2012), and Arias et al. (2018)
Thermal decomposition	Use organic iron compound as a precursor for oxidation reaction under nonaqueous and high-temperature condition	Wu et al. (2008) and Arias et al. (2018)
Sonochemical	Use precipitation reaction under high-intensity ultrasonication condition	Wu et al. (2008) and Arias et al. (2018)
Microwave-Assisted Synthesis	Use the microwave to assist the precipitation reaction	Wu et al. (2008) and Arias et al. (2018)
Electrochemical	Use reduction and oxidation reaction between anode and cathode cell for synthesis of nanoparticles	Ramimoghadam et al. (2014)
Flow injection technique	The reaction between iron precursor and alkaline medium occurs in a segmented flow tubular reactor	Salazar-Alvarez et al. (2006)
Pyrolysis method	Dissolve iron salt in a combustible solvent and spray metal solution through the flame for evaporation and burning of the solvent and the size of particle is dependent on the droplet size	Wu et al. (2008) and Eslamian and Shekarriz (2009)
Laser Ablation Synthesis in Solution (LASiS.)	Use laser beam that reaches the target bulk iron material immersed in liquid solution for ablation iron in the liquid solution	Arias et al. (2018) and Wu et al. (2008)

the extracellular environment, the cell membrane and intracellular particles. ROS are highly reactive oxygen species such as singlet oxygen, $^1O_2$; superoxide anion, $\bullet O_2^-$; hydroxyl radical, •OH; and hydrogen peroxide, H_2O_2. ROS can damage the essential bio-molecule of the cell. At the cell membrane, ROS are the cause of membrane leakage by peroxidation of lipid and modification of protein. At inner cell, ROS damage against lipid membrane and same mechanism as at membrane is applied. In addition, it can be damaging against DNA (Saleh et al. 2015).

Table 9.2 Biosynthesis and applications of IONPs

S. No.	Plant extract species	Characteristic (shape/size)	Biomedical applications	Reference
1	*Coriandrum sativum* leaf extract	Spherical/20–90 nm	Antioxidant, antimicrobial against *Micrococcus luteus*, *Staphylococcus aureus* and *Aspergillus niger*	Sathya et al. (2017)
2	*Punica granatum* peel extract.	–	Antibacterial against *Pseudomonas aeruginosa*	Irshad et al. (2017)
3	*Lantana camara* leaf extract	Nanorods/10–20 nm	Antibacterial against *Pseudomonas* sp. *Klebsiella* sp., *Staphylococcus* sp., *Salmonella* sp.	Rajiv et al. (2017)
4	*Eichhornia crassipes* leaf extract	Rods	Antibacterial against *Staphylococcus aureus*, *Pseudomonas fluorescens* and *Escherichia coli*	Jagathesan and Rajiv (2018)
5	Green tea and black tea leaves extracts	Spherical/green tea: 42–55 nm/black tea: 46–60 nm	Antibacterial against methicillin- and vancomycin-resistance *Staphylococcus aureus*. Antifungal against *Aspergillus flavus* and *A. parasiticus* Aflatoxin B1 adsorption activity	Asghar et al. (2018)
6	Tannic acid	Circular/10–30 nm	Antifungal against *Cladosporium herbarum*, *Trichothecium roseum*, *Penicillium chrysogenum*, *Alternaria alternata* and *Aspergillus niger*	Parveen et al. (2018)
7	*Zingiber officinales* rhizome extract	5–25 nm(Ag) and 1–3 nm(iron oxides)	MRI contrast agent, fluorescence agent, antibacterial against *Escherichia coli* and *Staphylococcus aureus*, antifungal against *Saccharomyces cerevisiae* and cell imaging	Ivashchenko et al. (2017)
8	*Rosmarinus officinalis* leaf extract	Spherical/20–80 nm	Cytotoxicity effect on cancer cell	Farshchi et al. (2018)
9	*Juglans regia* green husk extract	Average 5.77 nm	Cytotoxicity effect	Izadiyan et al. (2018)
10	*Citrus maxima* peels aqueous extracts	Irregular/10–100 nm	–	Wei et al. (2016)
11	*Ailanthus excelsa* leaves	Spherical/5–200 nm	–	Asoufi et al. (2018)

(continued)

Table 9.2 (continued)

S. No.	Plant extract species	Characteristic (shape/size)	Biomedical applications	Reference
12	*Moringa oleifera* extracts	Spherical/3.4–7.4 nm	Antibacterial against *Escherichia coli*	Katata-seru et al. (2018)
13	*Cynometra ramiflora* fruit extract	Spherical/58–78 nm	–	Bishnoi et al. (2018)
14	*Eichhornia crassipes* leaf extract	Spherical/20–80 nm	–	Wei et al. (2017)
15	Palm dates fruit extract	Irregular shape/5–40 nm	Antibacterial against *Escherichia coli* and *Staphylococcus aureus*, antioxidant	Al-Asfar et al. (2018)
16	Eucalyptus leaf extract	Irregular shape/80–90 nm	–	Gan et al. (2018)
17	Tea waste extract	Average 98.79 nm	–	Gautam et al. (2018)
18	Green tea and eucalyptus leaves extracts	Quasi-spherical/20–80 nm	–	Wang et al. (2014)
19	Green tea extract	Spherical/60–120 nm	–	Zhu et al. (2018)
20	*Lantana camara* extract	Spherical/average 21 nm	–	Nithya et al. (2018)

3. They release Fe^{2+} ion which chelate with inner and outer cellular ligands, for example, oxygen, phosphorus, nitrogen, and sulfur functional groups, affect to bio-molecule structure or function and impact to cellular homeostasis (Saleh et al. 2015; Slavin et al. 2017; Arias et al. 2018).

The antibacterial effect of biosynthesized IONPs against Gram-positive bacteria (*Staphylococcus* sp.) and Gram-negative bacteria (*Pseudomonas* sp., *Klebsiella* sp., *Salmonella* sp. and *Escherichia coli*) has been studied. IONPs have highest antibacterial activity against *Pseudomonas* sp. (Irshad et al. 2017). Moreover, it has an antibacterial effect against methicillin- and vancomycin-resistance *Staphylococcus aureus* (Asghar et al. 2018).

The antifungal effect of IONPs against *Aspergillus flavus*, *Aspergillus parasiticus Cladosporium herbarum*, *Trichothecium roseum*, *Penicillium chrysogenum*, and *Alternaria alternata* has been reported. IONPs have highest antifungal activity against *Trichothecium roseum* (Parveen et al. 2018).

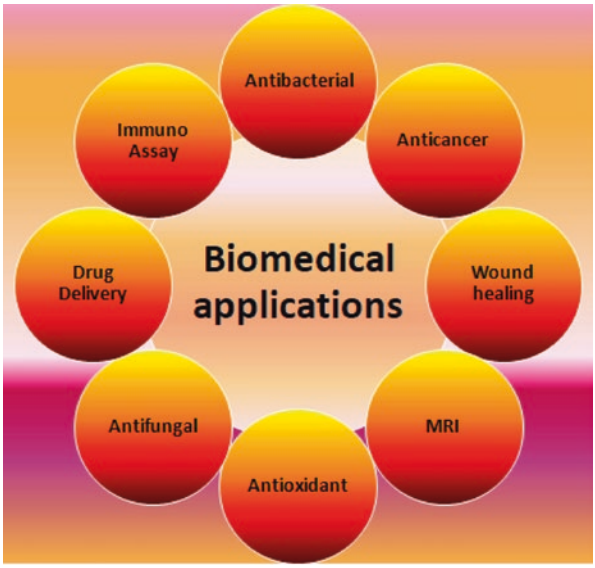

Fig. 9.2 Biomedical applications of magnetic nanoparticles

9.2.2 Anticancer and Cancer Theranostics

The magnetic properties of IONPs are as follows: (1) They are able to establish a local magnetic field. (2) They are able to generate thermal energy under alternating magnetic field condition which makes them suitable for cancer theranostics as a contrast agent and in cancer therapy as drug carriers or hyperthermia therapy (Collingwood and Telling 2016).

MRI is an extensive diagnostic technique for imaging inner organ or tumor by measuring the relaxation time of magnetization and changing of hydrogen in a water molecule in the body under a magnetic field. Hydrogen in each tissue has different changes in magnetization and relaxation time, so every anatomical structure gives a different picture. The contrast agent can affect change in the magnetization of hydrogen, enhance MRI sensitivity, and improve image visualization. And it can make a difference between normal tissue and cancer tissue for diagnostic by linking them with targeting ligands (Nedyalkova et al. 2017; Slavin et al. 2017). Contrast agent has two types, which include T_1 and T_2 types and we can separate type by their effect on hydrogen molecule. T_1 contrast agents modify the protons' longitudinal relaxation time (T_1), whereas T_2 contrast agents change the transverse relaxation time (T_2) of protons (Collingwood and Telling 2016; Nedyalkova et al. 2017; Nikitin et al. 2017). From studies of IONPs as contrast agent, every study found that they act as a T_2 type contrast agent (Ivashchenko et al. 2017; Nikitin et al. 2017).

Hyperthermia therapy is an injection of IONPs fluid directly into a tumor or blood vessel supplying tumor and navigating IONPs to tumor tissue and then alternating the magnetic field for heating IONPs in tumor tissue till it gets damaged and

Table 9.3 Example of IONPs application

S. No.	Application	Reference
1	Antibacterial activity – Against *Staphylococcus* sp., *Pseudomonas* sp., *Klebsiella* sp., *Salmonella* sp. and *Escherichia coli* – Against methicillin- and vancomycin-resistance *Staphylococcus aureus*	Irshad et al. (2017) and Parveen et al. (2018)
2	Antifungal activity against – *Aspergillus flavus*, *Aspergillus parasiticus Cladosporium herbarum*, *Trichothecium roseum*, *Penicillium chrysogenum* and *Alternaria alternate*	Asghar et al. (2018)
3	MRI contrast agent – IONPs are magnetic nanoparticles and act as T_2 contrast agent	Ivashchenko et al. (2017) and Nikitin et al. (2017)
4	Anticancer activity – IONPs have used as hyperthermia treatment and they were loaded into immunoliposomes for delivery them to target tumor cell	ITO (2004)
5	Drug delivery system – IONPs were modified on their surface and tested in absorb and release properties to mitoxantrone, the results have shown that IONPs have a strong absorption activity but lack desorption activity – This research is trial of conjugation of small molecule to dopamine-coated IONPs to increase their stability and minimize absorption of immune response – IONPs conjugated with cetuximab for the treatment of brain tumor by convection-enhanced delivery method. It can reduce the size of tumor	Zaloga et al. (2018), Freeman et al. (2018), Kaluzova et al. (2015), and Sherwood et al. (2017)
6	Wound-healing agent – Using Fe_2O_3-Ag-NPs combined with H_2O_2 can reduce the dose of H_2O_2 for dressing infected wound. So Fe_2O_3-Ag-NPs combined with low-dose H_2O_2 can increase the healing rate of wound	Yu et al. (2018)
7	Immunoassay – IONPs are used as signaling for generation of color in ELISA technique and to measure absorbance for quantitative samples	Zhang et al. (2017)

releases antigens or proteins for stimulating immune system against themselves (Toraya-Brown et al. 2014; Nedyalkova et al. 2017; Martinkova et al. 2017).

The efficacy of IONPs to anticancer effect and cancer theranostics depends on the amount of IONPs intake to the cancer cell. So, to increase the efficacy of IONPs by modifying of IONPs for improving specificity to target cancer cells, reduce the nonspecific uptake by the reticuloendothelial system (RES), prolong the blood circulation time, and evade macrophages. Surface modifications by conjugate targeting ligands can increase their cellular uptake into specific cancer cells. In addition, IONPs modified by PGA or PEG coating significantly reduce the macrophage cellular uptake (Li et al. 2017). Moreover, for modification of IONPs, the researchers

can use antibody immunoliposomes for content and delivery of IONPs to target tumor cells. For example, using anti-HER2 immunoliposomes-loaded IONPs for hyperthermia treatment against SKBr3 breast cancer cells showed that it can increase the amount of IONPs uptake to the breast cancer cells and also enhance the efficacy of heat generation of treatment (Ito 2004).

9.2.3 Vectors for Drug Delivery

Recently, IONPs were used for delivering drug because of their high surface area to the volume ratio and appropriate surface functionality to conjugate directly with drug or other molecules and modify their property to:

1. Lead drug to target site by linking nanoparticles with target ligands.
2. Control release of drug linked with a polymer which can absorb and release drug. These properties can increase the efficacy and reduce the side effect of the drug (Wilczewska et al. 2012).

IONPs can be used for delivery of anticancer drugs. IONPs modify their surface by getting lauric acid/albumin coated. The result has shown that IONPs have a strong absorption activity but lack desorption activity (Zaloga et al. 2018). Furthermore, IONPs were also conjugated with cetuximab for the treatment of brain tumor by convection-enhanced delivery method. The results have shown a reduction of tumor size in dogs (average over 50% reduction) and prolonged survival in rodents. Moreover, use of cetuximab-IONPs has been found to be more efficacious than cetuximab alone by greater cellular targeting and uptake, EGFR signaling alterations, EGFR internalization, and apoptosis induction in EGFR-expressing tumor cells (Freeman et al. 2018; Kaluzova et al. 2015).

Dopamine is a drug that gets coated on IONPs surface, to improve their properties, conjugation of the small molecule to dopamine coated IONPs (glutathione, cysteine, lysine, and Tris(hydroxymethyl) aminomethane). It can increase stability in solution-minimized absorption of serum proteins and increase their in vivo circulation time (Sherwood et al. 2017).

9.2.4 Wound-Healing Effect

Metal nanoparticles are used for wound dressing and for their antimicrobial effect against microbial pathogens in the wound because microbes in the wound are the main cause of inflammation and prolong the wound-healing period. Here, authors used Fe_2O_3-Ag-NPs combined with low-dose H_2O_2 for dressing the wound. Generally, H_2O_2 is used for dressing the wound for wound disinfectant, but it is harmful to healthy tissues and prevents wound healing. The reason is that Fe_2O_3-Ag-NPs act as peroxidase-like catalytic for H_2O_2, which can effectively

generate free radicals. So, they reduced the dose of H_2O_2 and increased the efficacy of wound-healing effect and reduce the toxicity of high-dose H_2O_2 to wound (Yu et al. 2018).

9.2.5 Immunoassay

Enzyme-linked immunosorbent assay (ELISA) is a popular immunoassay approach. It includes several types of technique. Zhang et al. (2017) used sandwich ELISA technique, which is a very high-specificity technique. A principle of this technique is using primary antibodies specific to the antigen of interest, coat them at the solid phase, and add biological samples. Then, detectible secondary antibodies are added, secondary antibodies are specific against antigen similar to primary antibodies. Finally, a color-changing substrate is added to generate a colorimetric signal, the intensity of which directly correlates with the level of the target antigen. IONPs are used as a signaling agent to generate signaling product from chelating reaction between Fe^{2+} and metal ion chelator bathophenanthroline disulfonic acid disodium salt (BPT) and used ascorbic acid for release of Fe2+ from IONPs. Chelating products between BPT and Fe^{2+} gave a red color. Finally, absorbance of colored product was measured by using a UV-Vis spectrophotometer (Zhang et al. 2017).

9.2.6 Neuroprotective Effect of Magnetic Nanoparticles

Several types of IONPs have been prepared and targeted against various neurodegenerative diseases such as Alzheimer's, Parkinson's, epilepsy, and depression. The pathogenesis of Alzheimer's disease is widely reported to be driven by the production and deposition of the β-amyloid peptide (Zverova 2019). Magnetic IONPs have the potential to interact with lysozyme amyloids in vitro leading to a reduction of the amyloid aggregates. The antiaggregating action of IONPs was reported due to the adsorption of lysozyme onto the nanoparticles (Bellova et al. 2010; Dulińska-Litewka et al. 2019) and the antiaggregating mechanism could be the possible reason for the anti-Alzheimer's effect of IONPs. The green synthesized IONPs using the aqueous extract of *Convolvulus pluricaulis* were found effective in improving oxidative stress, learning, and memory against scopolamine-induced amnesia in mice, suggesting the possible therapeutic role for the management of Alzheimer's disease (Lakshmi et al. 2017).

Implantation of IONPs along with external magnetic field exposure superparamagnetic IONPs in vivo against 6-hydroxydopamine (6-OHDA)-induced Parkinson's model significantly recovered the gait, postural balance, and improved the neuronal mitochondrial function (Umarao et al. 2016). In a study, dextran-coated IONPs improved the therapeutic efficacy of human mesenchymal stem cells (hMSCs) against 6-OHDA-induced Parkinson disease in mice. It has been demon-

strated that dextran-coated IONPs promote the hMSCs migration at the sites of degeneration of dopaminergic neurons and induce hMSCs differentiation into dopaminergic neurons at the diseased sites (Chung et al. 2018). The intraperitoneal injections of IONPs significantly improved the lipopolysaccharide-induced behavioral changes and depression in rats which were attributed to modulation of neurotransmitters and antiinflammatory effect (Saeidienik et al. 2018).

In the context of neuropeptide drug delivery, several peptides and their analogues have been used previously against various neurodegenerative diseases. However, they often failed to achieve the neuroprotective effect due to their inability in blood–brain barrier (BBB). Interestingly, in the oxidized state (Fe_2O_3), IONPs are stable, biocompatible, and effectively cross BBB; also it has been reported that such nanoparticles can be modified to carry peptide cargo (Minchin and Martin 2010; Jain 2012; Vinzant et al. 2017). Therefore, a neuropeptide in combination with nanoparticles as a drug delivery system has been tried. For instance, antianxiolytic effects were observed when IONPs conjugated with antisauvagine-30, a neuropeptide systemically administered against amphetamine withdrawal-induced anxiety in rats (Vinzant et al. 2017). Quercetin-conjugated SPIONs (QCSPIONs) are studied against diabetes-related learning and memory impairment in rats. In general, quercetin, a phytocompound has poor bioavailability and limited ability to cross the BBB (Ezhilarasan et al. 2014; Ebrahimpour et al. 2018). Quercetin in the form of QCSPIONs crossed BBB significantly compared with free quercetin. Further, QCSPIONs improved diabetes-related memory impairment induced by streptozotocin in rats (Ebrahimpour et al. 2018).

Interestingly, in a study, the neuroprotective potential of SPIONs against temporal lobe epilepsy (TLE) was studied. Mechanistically, TLE is a brain inflammation caused due to the induction of interleukin-1β (IL-1β) by activated glial cells. Therefore, anti-IL-1β monoclonal antibodies along with SPIONs were conjugated and administered intravenously. This combination readily crossed BBB and was concentrated in the astrocytes and neurons in epileptogenic tissues, rendered these tissues visible on magnetic resonance imaging (MRI), and simultaneously delivered anti-IL-1β monoclonal antibodies to the epileptogenic focus (Fu et al. 2016). This study showed that SPIONs could be a tool to analyze TLE via MRI and a novel approach to deliver antiinflammatory molecule in TLE. However, SPIONs at 50 and 100 mg/kg for 7 days oral administration in rats are shown to decrease the bodyweight significantly and therefore, biocompatibility and biosafety of SPION are to be considered (Najafabadi et al. 2018).

Undoubtedly, the IONPs have several beneficial properties as reported above. The possible neuroprotective effect of IONPs is depicted in Fig. 9.3. In contrast, studies have also shown the toxic properties of IONPs in the nervous system. Iron is involved in Fenton's reaction, a catalytic process that produces reactive oxygen species (ROS). Increased ROS is capable of causing significant oxidative stress that damage macromolecules and cellular organelles (Yarjanli et al. 2017; Ezhilarasan 2018). Iron accumulation in the nervous system is also responsible for apoptotic neuronal cell death and aggregation of Aβ and α-synuclein, which is said to play a dangerous role in the onset of Alzheimer's and Parkinson's diseases (Yarjanli et al.

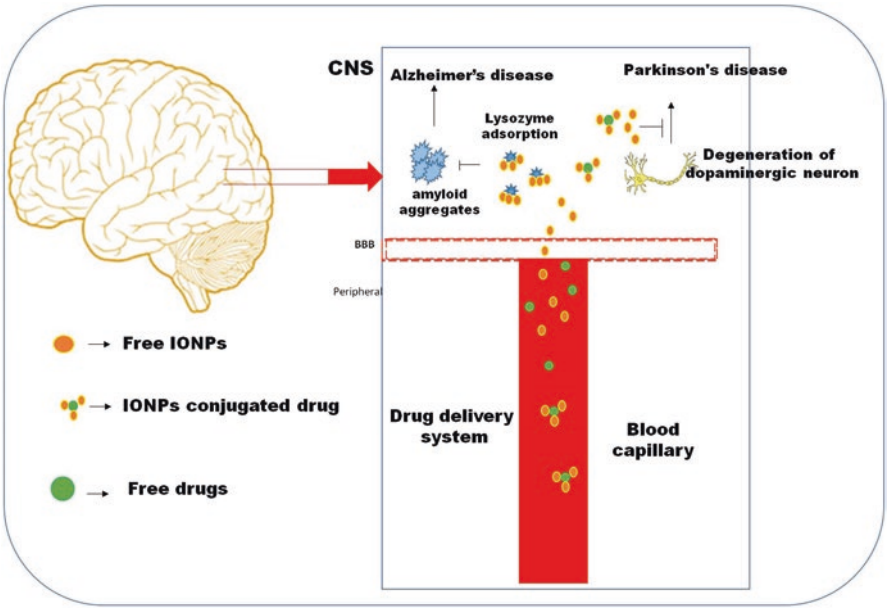

Fig. 9.3 Role of magnetic nanoparticles in Alzheimer's disease and Parkinson's disease

2017). Moreover, as a toxicity model, magnetic IONPs are used to induce oxidative stress-mediated mitochondrial dysfunction in the rat brain (Naserzadeh et al. 2018). These studies also claim that IONPs have toxic potential to the nervous system. Therefore, at this juncture, it is very difficult to postulate that IONPs are completely effective and safe as neuroprotective agents. The reason for these ambiguous results could probably be the differentiation in synthesis and fabrication of nanoparticles and their physiochemical properties such as size, concentration, surface charge, and the type of coating and functional groups of IONPs. The degree of IONPs induced toxicity depends on the fabrication and physiochemical properties of nanoparticles. Therefore, further studies are warranted on these lines.

9.3 Conclusion

This chapter has emphasized biosynthesis methods, biomedical applications of magnetic nanoparticles in general, and especially in neurodegenerative diseases. IONPs produced from plant extracts have several characteristics due to the difference of component in each plant extracts. Various biomedical applications are related to their properties. Due to the magnetic property, IONPs are used for cancer treatment and diagnostics. Their surface property makes them useful in drug delivery in neurodegenerative diseases. Nevertheless, IONPs also have physical and chemical properties suitable for other medical applications such as antimicrobial.

Therefore, IONPs can be the candidate material for future development in the biomedical field, particularly in neurodegenerative diseases including Alzheimer's disease.

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Chapter 10
Nanomedicines for Improved Antiretroviral Therapy in Neuro-AIDS

Aarti Belgamwar, Shagufta Khan, and Pramod Yeole

Abstract Human immunodeficiency virus is neurotropic which invades the central nervous system (CNS) in early course of systemic infection and makes the CNS an important dominant reservoir with the capacity to supply virus in low/undetectable viremia. Neuro-AIDS is the major upcoming issue among long-term seropositive survivors as a consequence of incompetence of antiretroviral in complete eradication of HIV from the CNS. Justification behind the low CNS concentration of antiretroviral is anatomical barrier and physicochemical properties of antiretrovirals. Some unmet needs in neuro-AIDS treatment are simplified CNS-targeted treatment regimen and disease-modifying therapies. Target-specific, safe, and controllable nanomedicines have been extensively studied, with particular success, to overcome the natural barriers to the antiretroviral drug delivery posed by the CNS anatomy, histology, and physiology. This chapter insight on current understanding of neuro-AIDS and the pathological mechanisms involved several limitations to the eradication of latent reservoirs and approaches to circumvent these limitations by state-of-the-art nanomedicines.

Keywords Neuro-AIDS · Antiretroviral · Nanomedicines · HIV · CNS

Nomenclature

ABC	ATP-binding cassette
ADC	AIDS dementia complex
AIDS	Acquired immunodeficiency syndrome

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ARV	Antiretroviral
BBB	Blood-brain barrier
BCRP	Breast cancer resistance protein
BCSFB	Blood-cerebrospinal fluid barrier
BMECs	Brain microvessel endothelial cells
BMVECs	Brain microvascular endothelial cells
cART	Combination antiretroviral therapy
CCR5	C-C chemokine receptor type 5
CD4	Cluster of differentiation 4
CNS	Central nervous system
CSF	Cerebrospinal fluid
CSFB	Cerebrospinal fluid-brain barrier
CXCR4	C-X-C chemokine receptor type 4
gp120	Glycoprotein 120
HAART	Highly active antiretroviral therapy
HAND	HIV-associated neurocognitive disorders
hCMEC/D3	Human cerebral microvascular endothelial cell line
HIV	Human immunodeficiency virus
NLCs	Nanostructured lipid carriers
PLA	Polylactic acid
PLGA	Poly(D,L-lactic-co-glycolic acid)
siRNA	Small interfering ribonucleic acid
SIV	Simian immunodeficiency virus
SLN	Solid lipid nanoparticle
Vpr	Viral protein R

10.1 Introduction

Human immunodeficiency virus (HIV), the primary cause of acquired immunodeficiency syndrome (AIDS), has triggered a devastating global pandemic with over 36.9 [31.1–43.9] million people living with HIV of which 75% [55–92%] of people suffering from HIV know their HIV status while about 9.4 million people are still unaware (Fact sheet 2018).

AIDS was first discovered as a new disease more than three decades ago on June 5, 1981, when increasing number of drug users and young homosexual men were dying because of opportunistic infections and malignancies due to progressive failure of immune system, and a causative agent was identified in 1983 as retrovirus HIV which is still provenience of significant mortality (Greene 2007). Majority of immune-depressed people are unaware of their condition, and for those who are aware the stigma and discrimination related to HIV remain major issue worldwide. Despite global programs of effective antiretroviral (ARV) therapy and prevention, epidemiological global data indicates adversity toward faster eradication of HIV

infection. Currently 21.7 million (19.1 million–22.6 million) people are accessing antiretroviral therapy. The United Nations Programme on HIV/AIDS (UNAIDS) proposed 90-90-90 target by 2020 which aims that 90% of HIV infected will know the status, 90% will receive access to antiretroviral therapy (ART), and 90% will be virally suppressed but as per latest factsheet of 2018 only 75-79-85 has been achieved.

Incompetence of antiretrovirals in complete eradication of HIV from the central nervous system (CNS) is the biggest issue in neuro-AIDS treatment. The HIV virus is neurotropic which invades the CNS in early stages of systemic infection and makes the CNS an important dominant reservoir owing to the restricted entry of anti-HIV drugs; hence, the brain is thought to form a viral sanctuary. This result in viral resistance and HIV-associated neurological sequel in the CNS constitute for neuro-AIDS.

Kramer-Hämmerle et al. (2005) reported that though the combination antiretroviral therapy (cART) plays a cornerstone role in the treatment of AIDS, complete eradication of HIV from this latent reservoir is the biggest issue in neuro-AIDS treatment mainly because of incapability of antiretrovirals to cross various biological barriers, poor absorption and limited oral bioavailability, long-term drug therapy, requirement of high dose and chronic therapy, lack of compliance, multidrug resistance maybe due to mutations in HIV, high plasma protein binding, substrate for efflux transporters, and powerful metabolic enzymes and transporters. The CSF/plasma ratio of currently available ARV is 0.002–0.63 which is unable to prevent latently proliferating virus even on prolonged therapy.

Currently, there are some unmet needs in neuro-AIDS treatment: (1) simplification of the daily treatment, (2) disease-modifying therapies, and (3) the CNS-specific targeting.

Nanomedicines have been extensively studied, with particular success, to overcome the natural barriers to the antiretroviral drug delivery posed by the CNS anatomy, histology, and physiology. The concept revolves around target-specific, effective, safe, and controllable drug delivery achieving higher concentrations of entrapped drugs with dramatically enhanced bioavailability. The present chapter will provide an insight into emerging trends in the nanomedicines for the treatment of HIV-related neurodegeneration.

10.2 HIV and Neuro-AIDS

AIDS is caused by two types of HIV lentivirus, viz., HIV-1 and HIV-2; both viruses are the result of multiple cross-species transmissions of simian immunodeficiency virus (SIV) naturally infecting African primates. Morison (2001) reported that HIV-1 is from SIV whose natural host is the chimpanzees *Pan troglodytes*. HIV-1 comprises of four distinct lineages, termed groups M, N, O, and P. Group M (major) was discovered first and represents pandemic, group O (outliers) was discovered in 1990 and is less prevalent, group N (novel) was identified in 1998 and even less

prevalent than group O, and group P was discovered recently in 2009 in a Cameroonian woman living in France. Group M strains are subdivided into nine subtypes (A, B, C, D, F, G, H, J, and K). HIV-2 has remained restricted to West African countries and is related to SIV from sooty mangabey monkey origin. It is less contagious as compared to HIV-1 and absence of mother-to-infant transmission (Sharp and Hahn 2012).

HIV transmission occurs mainly through sexual contacts, contaminated injectable equipment, from mother to child, and infected human body fluids (blood cells, semen, vaginal secretions, or breast milk).

HIV infection principally results in cell-mediated immune deficiency through progressive loss and dysregulation of CD4+ T lymphocytes, which weakens defense system against opportunistic infections and some types of cancer. This decline in lymphocyte level in the more advanced stage of infection is responsible for profound immune suppression characterized by advanced stage of AIDS (CD4 count of less than 200 cells/mm^3) which can take 2–15 years to develop depending upon the immunity of an individual (Connor et al. 1997).

10.2.1 Structure, Lifecycle of HIV, and Potential Targets of Antiretroviral Drug Activity

HIV is a retrovirus which belongs to the genus *Lentivirus* with size around 120 mm in diameter (Trkola 2004) as shown in Fig. 10.1. Presently there are more than 24 antiretrovirals approved by FDA to treat HIV infection classified into eight groups

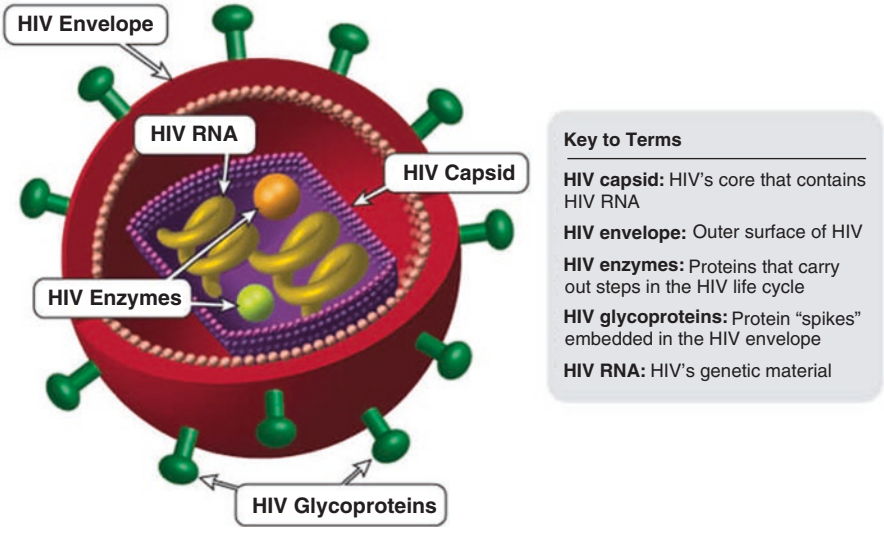

Fig. 10.1 Structure of an HIV virion. Figure reproduced from https://aidsinfo.nih.gov/understanding-hiv-aids/fact-sheets/19/73/the-hiv-life-cycle. Accessed 24 Apr 2019 with permission

Table 10.1 Classification of antiretroviral drugs

Class of antiretroviral	Name of drug
NRTIs: Nucleoside reverse transcriptase inhibitors	Abacavir, emtricitabine, didanosine, zalcitabine, stavudine
NtRTIs: Nucleotide reverse transcriptase inhibitors	Zidovudine, lamivudine, tenofovir
NNRTIs: Non-nucleoside reverse transcriptase inhibitors	Nevirapine, doravirine, efavirenz, etravirine, rilpivirine
PIs: Protease inhibitors	Saquinavir, indinavir, ritonavir, lopinavir, nelfinavir, amprenavir, fosamprenavir, atazanavir, tipranavir, darunavir
II: Integrase inhibitor	Raltegravir, dolutegravir
FI: Fusion inhibitor	Enfuvirtide
EI: Entry inhibitor	Maraviroc
Post-attachment inhibitors	Ibalizumab
Pharmacokinetic enhancers	Cobicistat

https://aidsinfo.nih.gov/understanding-hiv-aids/fact-sheets/21/58/fda-approved-hiv-medicines. Accessed 24 Apr 2019

(Table 10.1) according to their potential target site (Miller 2002; Rao et al. 2009). Figure 10.2 shows lifecycle of HIV and targets for antiretroviral drug activity.

10.2.2 Neuro-AIDS

Nearly 50% of HIV patients demonstrates neuropsychiatric complications as a result of HIV infection of the CNS which is called as neuro-AIDS (Koopmans et al. 2009). The HIV virus is neurotropic which invades the CNS in early stages of systemic infection and makes dominant reservoirs in the CNS. The CNS is a major target of HIV-1 infection and HIV-1-related diseases. Chronic HIV-1 infection of the CNS begins during primary infection and continues in nearly all untreated seropositive individuals. Late during the course of systemic infection, asymptomatic and seemingly benign CNS disease can progress to more severe stage. The clinical presentation is heterogeneous and can include a syndrome of cognitive, motor, and behavioral dysfunction formerly known as AIDS dementia complex (ADC), nowadays included in the collective term, HIV-associated neurocognitive disorders (HAND) (Zaitseva et al. 2003). A key feature of HIV populations in the CSF is that initially they may be identical to that in the plasma, but as the infection progresses the viral populations diverge, with the greatest divergence being observed in patients with HAND. In the late stages of AIDS, the CNS is also vulnerable to several severe opportunistic infections caused by bacteria, fungi, or other viruses, toxic effect of antiretroviral drugs, or HIV-associated malignancies. All these neurological conditions were initially associated with high morbidity and mortality.

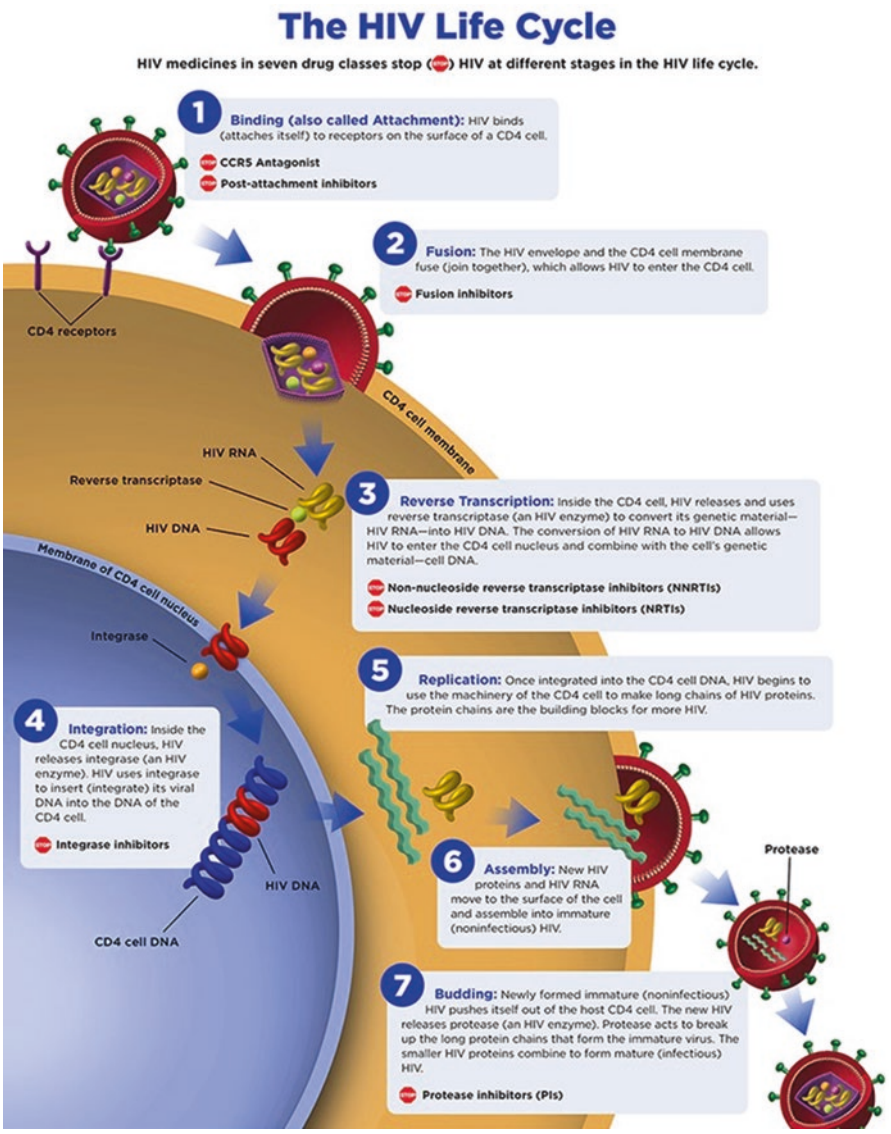

Fig. 10.2 Lifecycle of HIV and potential targets for antiretroviral drugs activity. Figure reproduced from https://aidsinfo.nih.gov/understanding-hiv-aids/fact-sheets/19/73/the-hiv-life-cycle. Accessed 24 Apr 2019 with permission

10.2.2.1 CNS as a Targets and Reservoirs of HIV

HIV targets the nervous and lymphoid systems as the major HIV receptors (CD4 and CD8), and several chemokine coreceptors (CXCR4 for lymphocytes and CCR5 for monocytes, macrophages, and microglia) located on their cellular surface which helps in the attachment of the virus to the cell and membrane fusion results in viral entry (Gonzalez-Scarano and Martin-Garcia 2005).

During early infection, HIV enters the CNS via infected immune cells such as CD4+ T lymphocytes, dendritic cells, and monocytes circulating in the blood and targets macrophages and microglial cells along with this non-CNS factor (periphery) which also contributes in initiating neurodegeneration and triggers dementia, for example, increased number of circulating monocytes having CD16 and CD69 receptors. HIV-activated cell progressively adheres to the endothelium membrane of the brain microvasculature, transmigrates, and, finally, triggers the process which loses integrity of blood-brain barrier (BBB) resulting in easy entry and replication of HIV inside the brain. BBB is composed of human brain microvascular endothelial cells, which are impermeable and tightly connected by intercellular junctions. Progressive HIV infection and immune suppression loses its integrity and permeability which leads to easy entry of toxins, free virus, and activated monocytes into the CNS (Kramer-Hämmerle et al. 2005).

Various virus-host cell interactions are responsible for HIV replication; intracellular environment plays a crucial role in virus replication. Infected cells can be differentiated with regard to the virus progeny they produce as productive (highly active/producers) and restricted (low/nonproducers). A productively infected cell contributes to transmission of infectious virus and generally leads to death of the infected cell, while cells that restrict HIV replication survive infection by suppressing production of virus progeny. Restrictedly infected cells establish virus reservoirs in which replication-competent viral genomes persist in a stable state and such virus reservoirs are responsible for rejuvenation of virus production with changes in the cell environment like elevated cytokine levels. The CNS is vulnerable for both types of infection (McGee et al. 2006).

10.2.2.2 Barriers of the CNS and the HIV Entry

Anatomically the CNS is safeguarded by three structural barriers, viz., the blood-brain barrier (BBB), blood-cerebrospinal fluid barrier (BCSFB), and the cerebrospinal fluid-brain barrier (CSFB). The BBB and BCSFB shield the brain from the periphery by means of tightly junctioned brain microvessel endothelial cells (BMECs) and choroid epithelial cells. These tightly packed structures possess very low and selective paracellular permeability while CSFB is less effective barrier as it is structured by loosely linked ventricular ependymal cells which allows reversible diffusion of solutes from ventricular cerebrospinal fluid (CSF) to brain parenchyma or vice versa (Johanson et al. 2011; Ballabh et al. 2004).

Many substrate-specific transporters such as monocarboxylate transport system, glucose transporter-1, insulin receptor, transferrin receptor, ceruloplasmin receptor, etc., are present on the BMECs. Some of these transporters have been shown to be involved in active transfer of drugs, nutrients, metabolites, hormones, and neurotransmitters across the BBB; they function in the direction of influx (from blood to brain) or efflux (from brain to blood). These efflux transporters are functional for detoxication and/or prevention of nonessential compounds and drugs from entering the brain. These influx-efflux receptors/proteins have been classified into two main groups, namely, ATP-binding cassette (ABC) transporters and solute carrier (SLC)

superfamily. Major ABC transporters and SLC carriers that affect drug delivery across the BBB are P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance-associated proteins (MRPs). Drugs, which are substrate for such efflux transporters, result in the reduction of efficacy in CNS, targeted drugs because of the lower delivery or decrease of CNS side effects for drugs that have pharmacological targets in peripheral tissues (Wong et al. 2012; Pardridge 2002). Solutes with sufficient lipid solubility having molecular weight up to 600 Dalton can passively diffuse transcellularly across the BBB, e.g., small lipophilic drugs, endogenous molecules, or certain neurotransmitters (Varatharajana and Thomas 2009).

The choroid plexuses as well as the arachnoid membrane constitute BCSFB, which is believed to be the secondary barrier that prevents drug penetration into the CNS. It controls flow of ions and molecules from the blood into the brain at the choroid plexus in ventricles.

Nevertheless, of this anatomical safeguarding, HIV-1 is able to overcome this tight barrier and invade the brain.

Three pathways have been proposed for HIV-1 entry into the brain at the BBB (Albright et al. 2003):

- Carriage of HIV from blood into the brain
- The "Trojan horse" hypothesis suggests that various infected immune cells such as monocytes and lymphocytes gain access to the brain by crossing BBB (Bell 2004).
- Passage of cell-free virus into the brain
- This is a possible mechanism of HIV entry in the brain; cell-free HIV particles from the blood could migrate paracellularly between BMVEC or by transcytosis in which BMVEC may take up HIV particles into vacuoles and release them to the brain side of the BMVECs. Entry of virus into BMVECs can lead to infection of the cell (i.e., presence of viral DNA) or transport of the virus particle through the cell into the brain or return of the virus to circulation.
- Transmission of virus by infected endothelial cells
- This possible pathway for HIV-1 entry into the CNS is the release of virus by infected, HIV-producing endothelial cells. Infected BMVECs may be capable of transmitting the virus to other cells by cell-to-cell contact, which may act as a reservoir for HIV.
- Entry of HIV-1 into the brain at the choroid plexus
- The choroid plexus is another potential gate of entry of HIV-1 from the blood into the brain. The choroid plexus is a highly vascularized structure involved in production of the cerebrospinal fluid from blood. Infection of cells of the choroid plexus may contribute to promulgation of HIV in the brain by CSF.

HIV can exit by following same pathways from brain to the periphery resulting in triggering reinfection.

After initial entry of HIV in the brain it triggers production of factors that are responsible for invasion of cells of the CNS. Figure 10.3 shows mechanism of neurodegeneration and neuroprotection in AIDS.

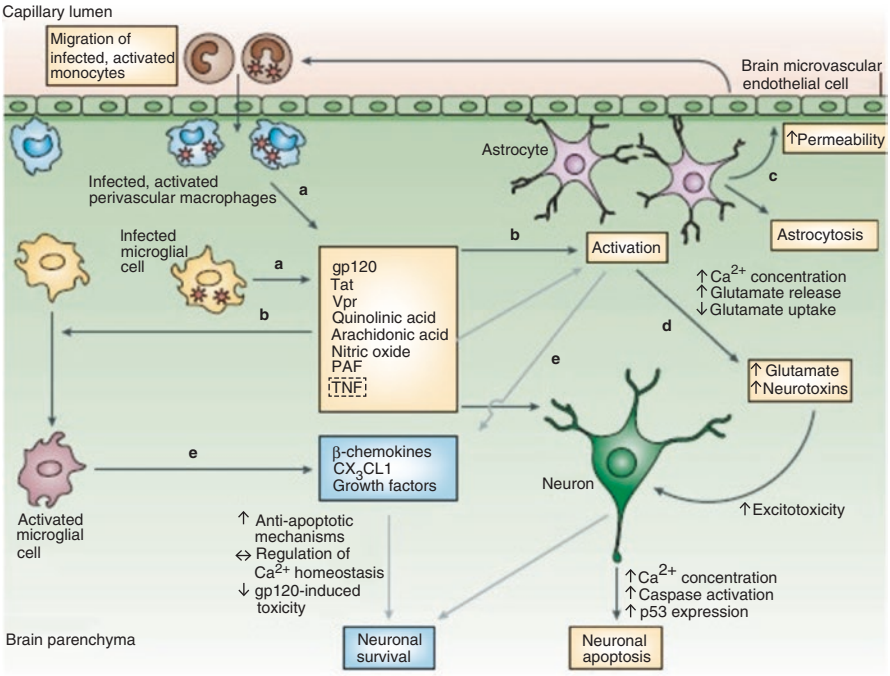

Fig. 10.3 Mechanisms of neurodegeneration and neuroprotection in AIDS. (**a**) Infected perivascular macrophages and microglia are responsible for producing HIV but might also release viral proteins that can be deleterious to the central nervous system. The HIV envelope protein gp120 (glycoprotein 120), Tat (transcriptional transactivator), and Vpr (viral protein R) have all been shown to be toxic in vitro to neurons and/or astrocytes, although their relevance in vivo is unknown. Infected and activated cells also produce other factors such as cytokines (including tumor necrosis factor, TNF), quinolinic and arachidonic acid, platelet-activating factor (PAF), and nitric oxide that are known to have neurotoxic effects. (**b**) Importantly, they promote the further activation (and to some extent, proliferation) of macrophages and/or microglia, as well as the proliferation and activation of astrocytes. (**c**) Activated astrocytes modify the permeability of the blood-brain barrier and promote the migration of more monocytes into the brain. (**d**) In addition, through increases in release of intracellular Ca2+ and glutamate and through decreases in glutamate uptake, the brain concentration of glutamate and other neurotoxins increases and results in excitotoxic death of neurons. (**e**) However, activation of macrophages and/or microglia, and TNF-mediated activation of astrocytes, also results in the release of β-chemokines, CX3C chemokine ligand 1 (CX3CL1), and growth factors, all of which are known to regulate Ca2+ homeostasis in neurons, to promote anti-apoptotic signaling pathways and to decrease gp120-mediated and excitotoxic neuronal cell death, thereby promoting neuronal survival. Gray arrows indicate neuroprotective pathways. Figure reproduced from Gonzalez-Scarano F and Martin-Garcia J (2005) The neuropathogenesis of AIDS. Nature Reviews Immunology 5:69–81 with permission from Springer Nature and Copyright Clearance Center

HIV-induced alterations of brain cell functions (Vyas et al. 2006) are as follows:

- Brain atrophy
- Infiltration of macrophages and lymphocytes
- Activation of microglia with formation of microglial nodules and multinucleated giant cells
- Widespread reactive astrocytosis
- Loss of specific neuronal subpopulations, loss of synapses and dendrites
- Loss of myelin surrounding neuronal axons (myelin pallor)
- Axonal damage
- Expression of HIV-1 proteins detectable with IHC

10.2.3 Shortcomings of Current Treatment

Combination antiretroviral therapy (cART) was a milestone in the history of HIV disease; also known as highly active antiretroviral therapy (HAART), it typically comprises of at least three antiretroviral drugs from two or more different antiretroviral classes which can efficiently inhibit HIV replication at several stages in the viral life cycle (Vyas et al. 2006). cART can decline plasma viral load below detectable level resulting in tenfold rise in life expectancy and has significantly improved the morbidity and mortality of HIV-seropositive patients. Undoubtedly, as a result of cART, this lethal disease has been transformed into a chronic pathology now but a concomitant rise in the other form of CNS dysfunction such as minor cognitive impairments/motor disorders has widely been noticed in the patients on HAART regimes. Cumulative neurodegenerative effect of HIV results in disturbed lifestyle in neuro-AIDS patients. Several problems exist with currently used therapy which further complicates the drug delivery to the CNS and increases incidence of the neuro-AIDS.

Hurdles in Neuro-AIDS Treatment

1. An anti-HIV drug may serve as substrate, inhibitor, or both for different influx-efflux transporters such as ABC transporters, P-gp, BCRP, and two SLC superfamily transporters, OCT-1 and 2. These carriers have been shown to affect the distribution of ARV drugs across the BBB.
2. Inadequate reachability of antiretrovirals across the BBB and BCSFB barriers has minimum effect on the resting viral loads in the brain hideout. This may result in gradual generation of resistance viral strain against HAART as has been seen in some of the infected populations.
3. These treatments are not targeted for inflammatory cascades underlying any of the HIV-associated neuronal disorders. Thus, HAART does not have direct effect on the HIV-associated inflammatory degeneration.

4. Antiretroviral drug's short half-life and limited bioavailability, due to extensive first pass metabolism including gastrointestinal degradation and high protein binding, may also add to their insignificant arrival in the CNS.
5. Moreover, emergence of various side effects; requirement of high dose, long-term chronic therapy; and high economic burden of cART may also result in cessation of patient compliance.
6. Nevertheless, little irregularity or interruption of cART treatment leads to resurgence of suppressed viral replication and so, challenge of complete restriction or elimination of progression of HIV infections still exists (Date and Destache 2013).
7. Elevated expressions of various enzymes such as γ-glutamyltranspeptidase, aromatic acid decarboxylase, alkaline phosphatase, etc., are found in cerebral microvessels. Metabolism-dependent luminal or abluminal expression of these enzymes significantly affects the dynamics and kinetics of antiretrovirals in the brain.

Overall, the basic problem of HAART failure in treatment of neuro-AIDS lies in the structural and functional complexity of brain barriers and physicochemical properties of antiretrovirals.

10.3 Nanomedicines in Neuro-AIDS Treatment

The ineffectiveness of antiretrovirals in neuroinfections and related disorders due to HIV demands patient care simple, CNS targeted, patient oriented, and effective in eradication of HIV from such latent reservoirs. Nanotechnology-based systems have been extensively studied, with particular success, to overcome the natural barriers to the antiretroviral drug delivery posed by the CNS anatomy, histology, and physiology. Particularly, the BBB represents an efficient obstacle to the delivery of many drugs. Nanomedicines in neuro-AIDS are designed to deliver drug by any conventional route in modified dosage with an intention to increase CNS concentration of drug. The concept of nanosystem revolves around target-specific, effective, safe, and controllable drug delivery achieving higher concentrations of entrapped drugs with dramatically enhanced bioavailability. They are known for their flexibility and versatility; they can be fabricated to offer various advantages:

- Reduces dose and shorten the therapy.
- Large surface to volume ratio of nanoparticles allows high drug loading and dissolution rate influencing the bioavailability.
- Protects drug molecules from harsh physiological condition.
- Manipulates undesirable physiochemical properties such as surface charge, biocompatibility, crystallinity, and hydrophobicity.
- Higher amount of drugs in nanoparticles leads in initial burst and then followed by slow release which modifies drug release kinetics and reduces dosing frequency.

- Delivers gene and/or immune-based therapeutics.
- Selective targeting can improve drug efficacy and reduce drug-associated toxicity and side effects.
- Can be molded for active or passive targeting.
- Enhanced effectiveness with minimum side effects.
- Multifunctionality can increase CNS targeting.
- Can be designed smartly to attain desirable therapeutic action using organic or inorganic materials.
- Nanomaterials can be explored for the development of novel drug delivery systems and redeveloping the current drugs delivery techniques to enhance the efficacy, patient compliance, ameliorated safety of drugs, and economical burden of healthcare system.
- They are able to protect the encapsulated drug from biological and/or chemical degradation and extracellular transport by P-gp efflux proteins. This would increase CNS availability of the drug.
- Their small diameter potentially allows nanoparticles to be transported transcellularly through olfactory neurons to the brain via the various endocytic pathways of sustentacular or neuronal cells in the olfactory membrane when delivered intranasally.
- Surface modification of the nanoparticles could achieve targeted CNS delivery drugs.
- Blocking drug efflux transporters at the BBB, allowing drug trafficking by nonspecific or receptor-mediated endocytosis, and increasing the local drug gradient at the BBB by passive targeting can assist in high drug targeting.

10.3.1 Nanocarrier in the Antiretroviral Therapy

Various nanocarriers used for the CNS drug delivery are shown in Fig. 10.4. Brief summary of preclinical nanosystems developed for antiretrovirals delivery in neuroAIDS is shown in Table 10.2.

10.3.1.1 Polymeric Nanoparticles

Polymeric nanoparticles are solid colloidal particles with size range from 10 to 1000 nm containing drug either dissolved, entrapped, encapsulated, and/or adsorbed or attached. Synthetic or natural polymers are important and well explored for nanodrug delivery system. These polymers exhibit a good potential for surface modification, provide good pharmacokinetic control, and are suitable for wide range of therapeutic agents. Polymers are gelatin, chitosan, poly(lactic-co-glycolic acid) copolymer, polylactic acid, polyglycolic acid, poly(alkylcyanoacrylate), and poly(methyl methacrylate). Polymers such as poly(butyl cyanoacrylate) (PBCA) and methyl methacrylate-sulfopropyl methacrylate (MMA-SPM) have been studied for delivery of zidovudine, lamivudine,

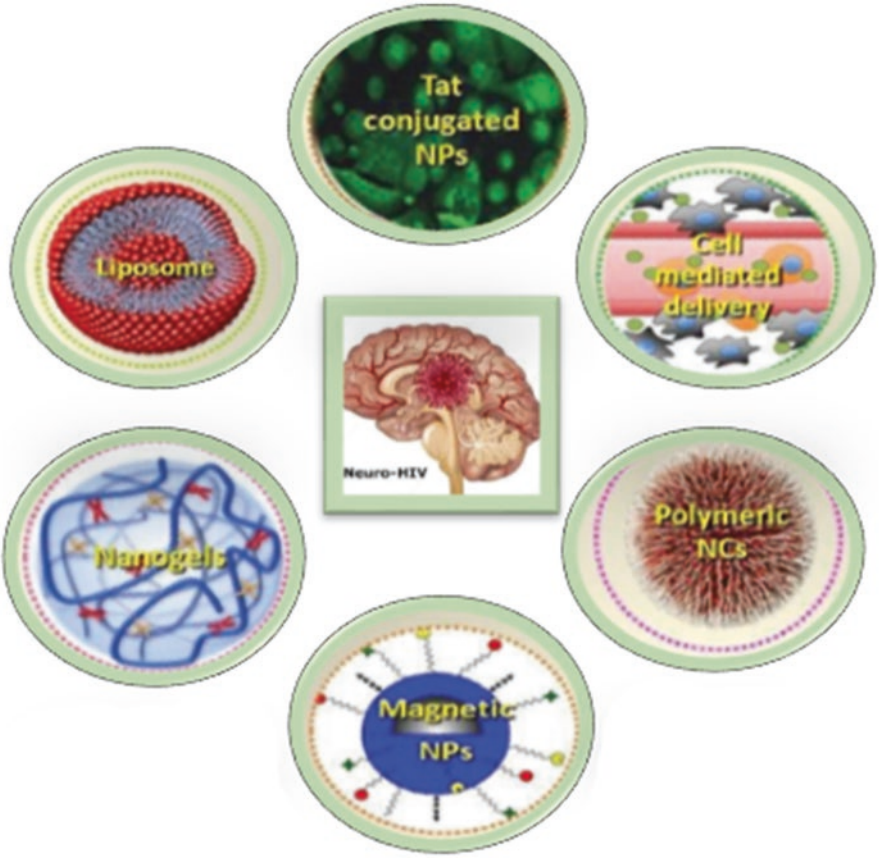

Fig. 10.4 Nanocarriers for the CNS drug delivery in neuro-AIDS

and stavudine and showed enhancement in vitro and in vivo drug levels. Kaur et al. (2008) reported delivery of stavudine, zidovudine, and lamivudine using MMA-SPM polymer nanoformulation increased in drug permeability (8–20-folds) across in vitro BBB model. Further same group developed SLNs (tripalmitin and cocoa butter mixture) of stavudine, delavirdine, and saquinavir and delivered across the in vitro BBB model and showed enhanced permeability of the antiretrovirals compared to free forms. Poly(D,L-lactic-co-glycolic acid) (PLGA) and polylactide (PLA) nanoparticle formulations also have been explored for delivery of various antiretrovirals (e.g., zidovudine (AZT), lamivudine) across mice brain (Mainardes et al. 2009). Same group developed biodegradable poly(L-lactide) (PLA) and poly(L-lactide)-poly(ethylene glycol) (PLA-PEG) blend nanoparticles containing zidovudine as a model drug. Nanoformulation was able to sustain drug delivery over time, but greater efficiency was obtained with PLA-PEG blend nanoparticles whose Tmax for this formulation was increased twofold compared with zidovudine from PLA nanoparticles and 16-fold compared with the zidovudine aqueous solution. In PLA and PLA-PEG nanoparticle formula-

Table 10.2 Brief summary of preclinical nanosystems developed for antiretrovirals delivery in neuro-AIDS

Drug	Nanosystems developed and key findings	Reference
Atazanavir	SLN permeated significantly studied using hCMEC/D3 cell line compared to aqueous solution	Chattopadhyay et al. (2008)
Amprenavir	Quantum rods effectively enhanced drug level in the brain	Mahajan et al. (2012)
3′-Azido-deoxythymidine-5′-triphosphate (AZTTP)	Transendothelial delivery of magnetic nanoparticles showed a comparable efficiency to the free drug in suppressing HIV replication. Magnetoliposomes showed sustained AZTTP release for 14 days with intact anti-HIV potency resulted in nearly threefold increase in vitro BBB transmigration when compared to free AZTTP	Saiyed et al. (2010)
Didanosine	Mannan-coated nanoparticles administered subcutaneously showed 12.4-fold increase in the brain drug level	Kaur et al. (2008)
	Significant drug level was observed when chitosan-loaded nanoparticles were administered intranasally	Alghananeem et al. (2010)
Efavirenz	Significant increase in cellular uptake of drug by Mo/Mac cells observed in mannose conjugated dendrimer which was 12 times higher than that of free drug and 5.5 times higher than that of t-Boc-glycine conjugated dendrimer	Dutta et al. (2008)
	Polymeric micelles of poly(ethylene oxide)-poly(propylene oxide) delivered by intranasal route have shown fourfold increase in CNS bioavailability of drug with respect to same system delivered intravenously	Chiappetta et al. (2013)
	Phenylalanine used as a transporter across BBB; conjugated phenylalanine SLN showed twofold to threefold and sevenfold to eightfold increase in brain accumulation compared to unconjugated SLN and drug alone, respectively	Vyas et al. (2006)
	Intranasal solid lipid nanoparticles showed 150 times more brain accumulation over orally administered marketed capsule	Gupta et al. (2017)
	Intranasal polymeric nanoparticles synthesized using chitosan-grafted HPβCD as a nanocarrier enhanced CNS bioavailability of drug 12.40-folds that of intravenous solution	Belgamwar et al. (2018)
Enfuvirtide	Drug-loaded iron oxide nanoparticles coated with PMA [poly(isobutylene-alt-1-tetradecene-maleic anhydride)] increased translocation by 170% across BBB within 3 h in in vitro studies	Fiandra et al. (2015)

(continued)

Table 10.2 (continued)

Drug	Nanosystems developed and key findings	Reference
Indinavir	Nanosuspensions of lipid nanocrystals were packaged into ex vivo cultivated bone marrow-derived macrophages and injected intravenously into severely immunodeficient HIV-1 encephalitis (HIVE) mice. High drug release fort 2 weeks with reduction in HIV replication	Dou et al. (2009)
	PEGylated nanoemulsions were studied for the brain delivery. Significantly enhanced CNS level of indinavir in brain for PEGylated emulsions (3.59 and 2.36 times, respectively) compared to drug solution and non-PEGylated emulsions	Kandadi et al. (2011)
Nevirapine	Albumin modified nanosuspension showed increased brain accumulation in rat of about 9.33 compared to unmodified	Shegokar and Singh (2011), Shegokar et al. (2011)
	In situ hybrid nanodrug delivery system was studied for size-dependent site-specific targeting ability to various viral reservoir sites in the body like brain, liver, etc. The results indicated 3.7-fold increase in the drug-loaded hybrid nanosystem accumulation in the brain when compared with drug solution	Jindal et al. (2017)
Ritonavir	P 85 micelles resulted 90-fold enhanced drug permeability across BBB	Batrakova et al. (1999)
	PLA nanoparticles conjugated with trans-activating transcriptor (TAT) peptides demonstrated 807-folds increase in brain drug level after 2 weeks of single intravenous administration in mice	Rao et al. (2009)
Saquinavir	Saquinavir encapsulated nanoparticles of poly(ethylene oxide)-modified poly(epsilon-caprolactone) were delivered to a THP-1 human Mo/Mac cell line. Higher uptake was noticed with the drug-loaded nanocarriers, compared to the free drug	Shah and Amiji (2006)
	Tf conjugated saquinavir quantum rods showed successful reduction in replication of HIV compared to unconjugated when tested using in vitro BBB model	Mahajan et al. (2010)
	Intranasal nanoemulsion showed significant enhancement in drug CNS bioavailability of drug	Mahajan et al. (2014)
Tenofovir	A layer-by-layer assembly of tenofovir and vorinostat (latency-breaking agent) on magnetic nanoparticles (MNPs) showed enhanced CNS drug level	Nair et al. (2016)

(continued)

Table 10.2 (continued)

Drug	Nanosystems developed and key findings	Reference
Zidovudine	PBCA nanoparticles grafted with CRM197 demonstrated drug delivery across BBB	
	Intravenously administered tuftsin-PEG-albumin nanoparticles resulted in enhanced uptake of about 21.1 against unconjugated	
	NLCs developed for brain delivery and showed significantly higher drug level in the brain cells	Joshy and Sharma (2012)
	Intranasal zidovudine-chitosan nanoparticles revealed promising approach for the incorporation of hydrophilic drugs for CNS targeting	Barbi et al. (2015)
Zidovudine-myristate (AZT-M)	Intravenously administered liposomes showed higher drug concentration as compared to unencapsulated drug	Jin et al. (2005)
Nevirapine and Atazanavir	Intravenous once a week nanosuspension was studied for antiretroviral and neuroprotective effect in humanized mice model and results showed 1000-fold reduction in viral loads	Dash et al. (2012)
Ritonavir, lopinavir, and efavirenz	PLGA nanoparticles administrated intravenously showed sustained drug level for 28 days compared to free drug	Destache et al. (2010)
Zidovudine and lamivudine	• Polybutyl cyanoacrylate (PBCA) nanocarriers hiked 8–20-fold and 10–18-fold drug delivery to the brain • Methyl methacrylate-sulfopropyl methacrylates (MMSPM) showed 100% increase in drug level in brain	
Stavudine, saquinavir, delavirdine	PS80-coated PBCA and MMSPM nanoparticles showed 12–16-fold and 4–11-fold increase in BBB permeability, respectively	Kuo and Su (2007)
Zidovudine, lamivudine, nelfinavir	Pluronic 85 (P 85) inhibits P-gp and increases ARV delivery	Spitzenberger et al. (2007)
Saquinavir, nelfinavir	P 85 increases BBB permeability of drug	

tions the mean half-life was increased by approximately 5.5 and 7 h, respectively, compared to aqueous solution. The relative bioavailability of zidovudine-loaded PLA and PLA-PEG blend nanoparticles was 2.7, relative to zidovudine-loaded PLA nanoparticles, and 1.3 relative to aqueous formulation. They observed that the size and surface charge are important parameters in a nanostructured system because these characteristics interfere directly in biological processes, such as transport across biological membranes and recognition by Mo/Mac and biodistribution.

10.3.1.2 Liposomes

Liposomes are lipid vesicles consisting of either one or more phospholipid bilayers with polar core for encapsulation of hydrophilic drugs and nonpolar phospholipid bilayer for amphiphilic and lipophilic drugs. Liposome-based nanomedicines offer cell specificity, low immunogenicity, and increased stability for effective targeting various anatomically difficult sites of the body such as the brain. Being lipophilic in nature, liposomes have successfully delivered various ARV drugs such as zidovudine, zalcitabine, and didanosine which have limited permeability to the CNS. Encapsulation of the antiretroviral drug, foscarnet within liposomes, resulted in a 13-fold increase in drug accumulation within the rat brains as compared to drug in solution (Dusserre et al. 1995). Due to their complex structural order, which occurs as a result of hydrophobic interactions, liposomes are relatively unstable in circulation (half-life ~4.2 h). The main disadvantage associated with liposomes is their lower plasma circulation time as they are rapidly removed from blood by the reticuloendothelial system leading to low CNS targeting. Surface modification with hydrophilic polymers like polyethylene glycol may enhance their blood circulation time by conjugating them to specific antibodies in order to improve their CNS targeting potential.

10.3.1.3 Dendrimers

Dendrimers are versatile and highly branched nanocarriers of 5–20 nm size investigated for the delivery of drugs across the BBB. Narrow molecular weight distribution and easy incorporation of targeting ligand are attractive features of dendrimers. Dendrimers have central core unit distinguished by their repeated branching structure emanating from the central core. The core of dendron is utilized for the entrapment of drug molecules for solubilizing of poorly water-soluble drugs, for controlled drug release, and for targeting/protecting drug from surrounding degrading environment. Dendrimers have interesting properties like uniform particle size, polyvalency of the end groups which helps in binding to diverse receptors, and ability to bind a variety of targeting agents to the high-density peripheral functional groups. Dutta et al. prepared G-5 PPI dendrimers to target efavirenz (Dutta et al. 2008) and lamivudine (Dutta and Jain 2007) to target human monocytes/macrophages. Lamivudine-loaded mannosylated polyamidoamine dendrimers were evaluated for their in vitro antiretroviral activity in HIV-infected MT2 cells; results revealed that lamivudine dendrimers exhibited 21-fold higher drug uptake when compared to drug in solution. The major limitations of dendrimers are the variability of release mechanisms and the short term of release kinetics for dendrimer-based drug delivery platforms. Drugs encapsulated within dendrimers tend to be released rapidly, expelling their payload prematurely before the macromolecules can reach their target sites.

10.3.1.4 Polymeric Micelles

Polymeric micelles are nanoshell-like structures which are prepared from amphiphilic block copolymers. Having unique properties of smaller size and higher drug solubilization makes micelles a promising candidate for drug and protein delivery across BBB. The three most widely studied block copolymer classes are characterized by their hydrophobic blocks and are poly(propylene oxide), poly(L-amino acid), and poly(ester)s. The polymeric micelles used for drug delivery have shown the decreased toxicities, enhanced targeted drug delivery, and improved therapeutic efficacy of active pharmaceutical ingredients (Dhembre et al. 2011). Kabanov and Alakhov (2002) examined the influence of Pluronic P85 on the permeability of a broad range of antiretrovirals by using bovine brain microvascular endothelial cell (BMVEC) model; results showed 19-fold increases in the drug permeability compared to free drug solution. Batrakova and co-workers investigated the co-administration of zidovudine, nelfinavir, and lamivudine with P85 and reported an improvement of the drug permeability in vitro in BMVECs and macrophages. In in vivo studies, increased drug delivery to the brain of wild-type mice was observed, but results were opposite in mdr1a/b knockout mice, showing that the drug permeability effected by Pluronic is facilitated in part by P-gp inhibition at the BBB. Though micelles offer so many promising advantages, its biological stability, lower rate of drug dissociation, and long drug retention time limit their clinical use for CNS drug delivery (Batrakova et al. 1999).

10.3.1.5 Solid Lipid Nanoparticles (SLNs)

Solid lipid nanoparticles have emerged with tremendous potential to be used as a nanodrug carrier for hydrophilic and lipophilic drugs. For the synthesis of SLN, one or more biocompatible solid lipids such as fatty acids, glycerides, waxes, glycerine mixtures, etc., are liquefied by heating, dispersed, and stabilized in either ionic or nonionic surfactant. SLNs offer distinct advantages such as high drug loading capacity, feasibility of incorporating both hydrophilic and hydrophobic drugs, drug release which can be controlled, high permeability, enhanced drug bioavailability and less toxicity, feasibility for large-scale production, and suitable for sterilization process which are most important features of these lipids, and these lipids are safe, biocompatible, and biodegradable. Main disadvantage of SLNs is its instability, as drug molecules positioned in between the fatty acid chains or as amorphous clusters in crystal imperfections within SLN matrix, and when lipid transforms to low energetic form, it forms a perfect crystalline lattice that allows very small space for the drug molecules. This change leads to expulsion of encapsulated drug molecules during storage, especially when SLN matrix is composed of a highly purified lipid, which leads to limited drug loading capacity of SLNs (Blasi et al. 2007). Kuo and Su (2007) used in vitro BBB model of human BMECs and demonstrated that the permeability coefficient of stavudine, delavirdine, and saquinavir loaded on SLN

was respectively 4–5, 8–11, and 9–11 times higher as compared with free drugs. Chattopadhyay et al. (2008) showed a significantly improved cellular uptake of SLN-loaded atazanavir compared to aqueous solution in in vivo studies carried out on BBB model. Similarly, higher cellular accumulation of Rhodamine-123, a substrate of efflux transporter P-gp, was also shown in this study. Thus, it was predicted that SLN may either mask or bypass the efflux pump.

10.3.1.6 Nanostructured Lipid Carriers

Nanostructured lipid carriers (NLCs) overcome some of the drawbacks of SLN like limited drug loading, bursting of loaded drug molecules, etc. Das and his co-worker have clearly shown that NLCs are much more stable than SLNs (Das et al. 2012). NLCs are formulated with a mixture of solid lipid and liquid lipid; a blend of a liquid and solid lipid creates a less perfect crystalline structure with many imperfections providing thus more space for drug accommodation. Lipids which are insoluble in room temperature as well as in body temperature are used as the solid lipid component, e.g., triglycerides (tristearin, tripalmitin, trimiristin), fatty acids (stearic acid, palmitic acid), waxes (carnauba, cetyl palmitate), etc., while lipids, which are liquid at room temperature, are used as the liquid lipids; examples include medium chain triglycerides, oleic acid, isopropyl miristate, etc. (Doktorovováa et al. 2010). NLCs are colloidal carrier systems that can penetrate/permeate the membrane effectively and by optimizing ratio of lipid matrix composition, drug can be delivered for prolonged or controlled release with decreased burst effect (Hu et al. 2006). Joshy and Sharma (2012) developed and evaluated zidovudine NLCs for brain delivery and significantly higher drug level in the brain cells was observed. As NLCs show versatility, currently majority of research work is focused on developing of NLCs for brain targeting.

10.3.1.7 Magnetic Nanoparticles

Magnetite (Fe3O4) and maghemite (Y-Fe2O3) are the most commonly used magnetic nanoparticles (MNPs) in the field of biomedicine and have been extensively investigated for targeted drug delivery. Nanoparticles under the influence of external magnetic force are directed to deliver drug to the brain. Thus, techniques like magnetic resonance imaging and magnetometry can be applied for indirect measurement of localization of nanoformulation. Saiyed et al. (2010) have investigated the transendothelial delivery of magnetic nanoparticle-bound antiretroviral drugs 3′-azido-3′-deoxythymidine-5′-triphosphate (AZTTP); nanoformulation showed a comparable efficiency to the free drug in suppressing HIV replication. An AZTTP magnetoliposomes were developed which showed sustained AZTTP release for 14 days with intact anti-HIV potency which resulted in nearly threefold increase in vitro BBB transmigration when compared to free AZTTP and did not affect the BBB integrity. Nair et al. (2016) demonstrated a layer-by-layer assembly of 10 of

ovirand vorinostat (latency-breaking agent) on magnetic nanoparticles (MNPs). Fiandra et al. (2015) successfully enhanced the permeation of enfuvirtide (T-20) iron oxide nanoparticles coated with poly(methacrylic acid) amphiphilic polymer.

10.3.1.8 Surface-Modified and Cell-Based Nanovehicles

The inherent migratory potential of inflammatory response cells toward the zone of inflammation can be exploited for the targeted drug delivery. Drug-loaded nanovehicles are either packaged inside the cell or attached to the cell surface for the delivery at the specific injury site. Thus, coating of nanocarriers with the receptor-specific moieties such as mannose, folate, gelatin, A-protein, RGD peptide, etc., complements the recognition by specific cell surface receptors leading to cellular internalization.

Dou et al. (2006, 2009) demonstrated that macrophage-based nanoparticle platform can successfully deliver the active ARV drug in the brain. Indinavir formulated in suspensions of lipid nanocrystals was packaged into ex vivo cultivated bone marrow-derived macrophages and injected intravenously into severely immunodeficient HIV-1 encephalitis (HIVE) mice. High drug release in different regions of the brain was noticed consistently for at least 2 weeks and corresponding reduction in HIV replication was observed in the HIVE brain regions. Jain et al. (2009) coupled the gelatin nanoparticles with mannose (MN-GNPs) using a two-step desolvation technique for the selective delivery of didanosine to the target organs. The results of the study depicted that the unconjugated gelatin nanoparticles (G-NPs) released a comparatively higher percentage of drugs than MN-G-NPs. But cellular uptake by MN-G-NPs was 2.7 times higher than G-NPs. Hence, coupling of the nanoparticles with mannose significantly enhanced the lung, liver, and lymph node uptake of drug following administration of MN-G-NPs in comparison to uncoupled G-NPs or free drug. Rao et al. (2009) attempted to reduce the viral pool in CNS, by formulating trans-activating transcriptor (TAT) peptide-conjugated polylactic acid nanoparticles to bypass the efflux action of P-glycoprotein and to increase the transport of the encapsulated ritonavir across the blood-brain barrier to the CNS. It was found that the brain drug level with conjugated particles was 800-fold higher than that with drug in solution after 2 weeks and TAT-conjugated particles maintained therapeutic drug levels in the brain for sustained period.

10.3.1.9 Prodrug and Conjugate Strategies

Prodrugs are modification of those drugs that are activated by undergoing transformation in vivo to form the active drug. This approach is developed for those drugs which are chemically unstable, having extensive first pass metabolism, and suffers from low solubility and low bioavailability problems. In ARV category fosamprenavir (prodrug of amprenavir) and tenofovir disoproxil fumarate are the two prodrugs that have been developed till date which showed improvement in GIT

absorption but no ARV has been studied for brain-targeting approach (Rautio et al. 2008).

10.3.1.10 Miscellaneous

Miscellaneous nanoparticles used for the ARV delivery to the brain include nanogels, nanosuspensions and nanoemulsions, etc.

Nanogels are designed to deliver both hydrophilic and hydrophobic drugs as they offer various interesting properties such as excellent biocompatibility and degradability profile, biological stability, swelling property in aqueous media, higher drug loading capacity, low particle size, electromobility, and a nonimmunologic response that makes them a robust strategy for drug delivery in neuro-AIDS. NRTIs such as zidovudine and didanosine have been delivered using this technique (Vinogradov et al. 2010). Shegokar et al. (2011) prepared nevirapine nanosuspensions by high-pressure homogenization method; it was further surface modified with PEG 1000, serum albumin, and dextran 60. It was observed that albumin-modified nanosuspension showed an increased accumulation in the brain of rats (Shegokar and Singh 2011; Shegokar et al. 2011). Dash et al. (2012) studied the antiretroviral and neuroprotective effects of nevirapine and atazanavir nanosuspension in humanized mice model and viral loads were found to be reduced by 1000-fold after once a week intravenous administration (Dash et al. 2012). PEGylated nanoemulsions of indinavir were studied by Kandadi et al. (2011) for the brain delivery. The results showed a significantly enhanced CNS level of indinavir in the brain for PEGylated emulsions (3.59 and 2.36 times, respectively) compared to drug solution and non-PEGylated emulsions, at all time points studied (Kandadi et al. 2011). Saquinavir-encapsulated nanoparticles were prepared using poly(ethylene oxide)-modified poly(epsilon-caprolactone) by means of a solvent displacement technique and were delivered to a THP-1 human Mo/Mac cell line to improve higher intracellular drug level. A significantly higher uptake was noticed with the drug-loaded nanocarriers, compared to the free drug (Shah and Amiji 2006). Jindal et al. (2017) prepared in situ hybrid nanodrug delivery system of nevirapine to study the size-dependent site-specific targeting ability of the delivery system to various viral reservoir sites in the body like brain, liver, etc. Biodistribution studies were carried out and the results indicated a 3.7-fold increase in the accumulation of the drug-loaded hybrid nanosystem in the brain when compared with plain nevirapine solution administered intravenously.

Rodriguez et al. (2017a) formulated and evaluated ferric-cobalt electromagnetic nanomaterial bounded to Beclin1-siRNA for brain delivery. This system effectively reduced the replication of virus in the brain and associated inflammatory responses in the microglial cells. The nanoformulation showed promising results when tested for its effect on neuronal viability; no significant neuronal death was observed after 72 h. Another study was conducted using cationic linear polyethylenimines (PEI) as the carrier for the small interfering RNA (siRNA) against the Beclin1 gene, administered intranasally. The biodistribution of the formulation was studied using fluorescein isothiocyanate as fluorescent marker. No significant adverse reactions were

observed and the cytoplasm of neuronal and glial cells showed the presence of siRNA even after 24 h after administration of the PEIsiRNA nanocomplex (Rodriguez et al. 2017b).

Das and Chakraborty (2015) in their review discussed various invasive and non-invasive strategies for brain targeting. Invasive strategies such as alteration of BBB permeability by osmotic and biochemical disruption might be risky for CNS as passage of unwanted components may occur. Other invasive techniques include intracerebral implants or intraventricular infusion of drugs but such techniques cannot be applied for long-term therapy in neuro-AIDS treatment. Noninvasive approaches include intranasal drug delivery via olfactory pathway, targeting brain with MRI-guided focused ultrasound for targeted localized effect and cell-gene targeting system.

Intranasal nanomedicine delivery is a new avenue to target the CNS for preventing or treating this silently spreading neuroinfection. Nasally administered drugs may reach the CNS via olfactory, trigeminal, or systemic pathway. Literature indicates successful delivery of antiretrovirals by intranasal routes; Barbi et al. (2015) prepared zidovudine-chitosan nanoparticles; the study revealed that the positive surface of the nanoparticles is very important for the mucoadhesive properties due to interaction with the sialic groups of the mucin. Nuclear resonance magnetic data showed that the higher concentration of chitosan in the nanoparticles favored the interaction of few phosphate units (pyrophosphate) by ionic interaction. In vitro permeation study showed that the nanoparticles promoted an increase in the flux of the drug through the nasal mucosa. In view of these results, chitosan nanoparticles were found to be a promising approach for the incorporation of hydrophilic drugs. Dalpiaz et al. (2015) conjugated zidovudine to ursodeoxycholic acid to produce prodrug which remained in murine macrophages 20 times higher than zidovudine, and designed chitosan chloride microparticles had more CSF uptake in rat; saquinavir mesylate nanoemulsion was developed by Mahajan et al. (2014); the nanoemulsion showed a significant increase in drug permeation with higher drug concentration in the brain after intranasal administration conclusively demonstrated transport of drug in the CNS at larger extent after intranasal administration as nanoemulsion. Belgamwar et al. (2018) delivered efavirenz polymeric nanoparticles intranasally by using newly synthesized chitosan-grafted-HPBCD copolymer as a nanocarrier. The CNS bioavailability of drug was found to be increased by 12.40-folds that of i.v. solution. Further same group delivered dolutegravir-loaded HPBCD nanoparticles intranasally and successfully enhanced CNS bioavailability in the neuro-AIDS (Belgamwar et al. 2019).

10.4 Conclusion and Future Perspectives

Neuro-AIDS remains a major challenge among the AIDS survivors, though the introduction of cARV was a major breakthrough in improving life span of AIDS patients but quality of life is severely disturbed due to neurological complications as

a result of HIV infection and several other opportunistic infections. In recent years, scientific community has given tremendous efforts in understanding the mechanism and progress of neuropathogenesis but long-term treatment schedules of HAART successfully suppressing HIV from peripheral tissues fail to combat latently proliferating viral reservoirs of the CNS. Justification behind this insufficient passage of ARV is due to anatomical barrier offered by the CNS and physicochemical properties of drugs. Thus, the CNS continues to act as an important sanctuary site for HIV virus suggesting an alarming need to develop successful therapeutic strategies for CNS targeting.

In essence, there are evidences suggesting that nanomedicines can be designed and developed for successful transport of ARVs across the CNS barriers in neuro-AIDS. Various approaches such as polymeric nanoparticles, SLN, liposomes, nanomicelles, magnetic nanoparticles, and surface-modified nanoparticles can be delivered conveniently by noninvasive technique for enhancing high CNS bioavailability of ARVs.

Research scientist involved in charting the future evolution of neuro-AIDS therapeutics should focus on making neuro-AIDS patient's care more streamlined, patient oriented, and effective to eradicate HIV from latent reservoirs to achieve zero. Though novel nanomedicines have potential to address the limitations of cART, still there is much room for innovation as the CNS infection and HIV involvement in neuro-AIDS is a complex obscurity and needs to be explored extensively. There is a need of simple in vitro models to understand mechanism of neuroinvasion and neuropathogenesis which will assist to increase potential of nanomedicines in drug targeting. Novel methods should undergo ex vivo and in vivo studies to ascertain the effectiveness of developed method in neuro-AIDS patients. Successful product development and delivery for the CNS targeting and translational studies in humanized murine model will be a novel way to assess efficiency in eradication of HIV-1 virus from CNS. Majority of research is on laboratory scale; efforts are needed to extrapolate research toward cure trials. There is a need to execute successful clinical trials of developed products as per the standards of international agencies. Development of prognostic markers will help clinicians for early diagnosis of neuro-AIDS. Quantitative method for neurocognitive impairment should be developed to understand prevalence. Emphasis should be made on development of theranostic nanomedicines which will guide and target latent reservoirs.

Neuro-AIDS image monitoring and management program should be developed for drug delivery and release mechanism, disease progression, brain mapping, drug efficacy, and neurobehavioral assessment. Smart easy assay system should be developed to monitor viral load and ARV. Significant research in painless therapy will be new avenue in treatment of neuro-AIDS with increased patient compliance to prevent drug resistance and relapses of viral load.

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Chapter 11
Nanocarrier-Mediated Drug Delivery Systems for Neurodegenerative Diseases

Sathika G. G. Arachchige, Ryan Rienzie, and Nadeesh M. Adassooriya

Abstract The central nervous system (CNS) is affected in neurodegenerative diseases which lead to neuronal malfunction and death. The blood-brain barrier (BBB) which separates blood from the brain maintains the homeostasis in the CNS. It shows permeability only for selected substances, especially molecules which are small in size. Hence, the entry of certain drugs required for the treatment of neurodegenerative diseases is prevented. Mostly, less than 1% of drugs cross the BBB resulting in low efficiency in conventional treatment methods. The advent of nanotechnology for the treatment of neurodegenerative disease serves as a promising alternative approach. The most interesting fact is nanocarriers can be used to cross the BBB efficiently enabling them to use in targeted drug delivery. In here, drugs are combined in nanomaterials which facilitate the drug delivery across the BBB and size, type, polarity, and surface chemistry of nanoparticles are the determinants of efficacy in transporting across BBB. Nanocarriers can be designed in such a way that it does not alter the effect of the drugs. However, care has to be taken when choosing nanomaterials (NM) as some are nonbiodegradable. They can accumulate in the brain and cause toxic side effects. The fate of such particles is yet to be discovered. This chapter extensively discusses the nanocarrier-mediated drug delivery for the treatment of these diseases and their future prospects.

Keywords Central nervous system · Blood-brain barrier · Cell death · Nanocarriers · Targeted drug delivery · Carrier-mediated specificity · Biodegradable

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Nomenclature

AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
BBB	Blood-brain barrier
BCSFB	Brain cerebrospinal fluid barrier
CED	Convection-enhanced delivery
CNS	Central nervous system
HD	Huntington's disease
HTT	Huntingtin
LBD	Lewy body dementia
MSNPs	Mesoporous silica NPs
NC	Nanocomposite
NM	Nanomaterials
NPs	Nanoparticles
PBCA	Poly(butylcyanoacrylate)
PD	Parkinson's disease
PEG	Poly(ethylene glycol)
PEI	Polyethylenimine
PLGA	Poly(lactide-co-glycolide)
PNP	Polymeric nanoparticles
siRNA	Short-interfering RNA
SLNPs	Solid lipid nanoparticles
VD	Vascular dementia

11.1 Introduction

Neurodegenerative diseases are caused by neuronal malfunction and death in different regions of the nervous system. Neurodegenerative diseases include Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), Lewy body dementia (LBD), amyotrophic lateral sclerosis (ALS), prion disease, vascular dementia (VD) and Pick's disease, etc. (Spuch et al. 2012; Goyal et al. 2014). Emergence of neurodegenerative diseases and disorders in the central nervous system (CNS) occur for half of human population when they reach the age of 70 years (D'Souza 2019).

The BBB and the brain cerebrospinal fluid barrier (BCSFB) protect the CNS system from pathogens and toxins (D'Souza 2019); hence, it protects the brain from many diseases. BBB is highly selective for the penetration of molecules; thus, it restricts the movement of substances such as hydrophilic compounds, charged molecules, and small proteins from the blood plasma to the CNS (Lockman et al. 2002), maintaining a constant environment in the brain. Due to these restrictions of the BBB, most therapeutic agents are unable to reach the CNS, limiting the treatment for neurodegenerative diseases (Patel et al. 2012).

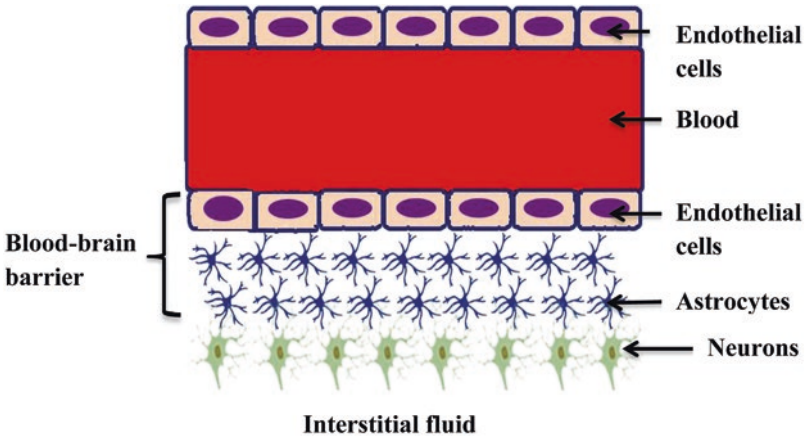

Fig. 11.1 Schematic representation of the blood-brain barrier (BBB) (Modified from D'Souza 2019)

The BBB has a contiguous layer of specialized capillary epithelial cells joined by tight junctions on one side and astrocytes with foot processes on the other side; hence, it separates blood from the brain (Fig. 11.1) (D'Souza 2019). The barrier has similar properties as a continuous cell layer; hence, lipid-soluble molecules can only be transported across the cell layer whereas the entry of hydrophilic substances is limited (Lockman et al. 2002). Several attempts have been made to overcome the limitations of BBB due to its specific and restrictive nature, either by changing the characteristics of the barrier or the drug. The tight junctions at the BBB can be opened using several approaches. However, opening of the barrier also allows the entry of toxins and unwanted molecules, significantly damaging the CNS (Greig 2011). Since altering the permeability of the BBB is often associated with undesirable consequences, researchers have attempted changing the drug molecules enabling them crossing the BBB. In here, the drug molecule can be designed in such a way that it becomes more lipid soluble (prodrugs); thus, it can penetrate the CNS. However, all the compounds cannot be manipulated in this way. Also, pharmacokinetic parameters may be altered due to the enhanced lipid solubility leading to unfavorable clearance and half-life (Greig et al. 1990). Carriers can be used as an alternative mean of delivering drugs to the CNS. However, the drug molecules must have carrier-mediated specificity. The NPs do not require drug molecular specificity and the nanomaterial carrying the drug may penetrate the BBB (Lockman et al. 2002). Therefore, nanoparticles (NPs) are ideal to be used in the drug delivery system to cross the BBB as changing the characteristics of the barrier or the drug molecules is not necessary (Lockman et al. 2002).

11.2 Advent of Nanotechnology

Nanotechnology is an interdisciplinary technological approach that has been greatly influenced over a range of fields including medicine, food and agriculture, and energy that offers a greater impact to uplift the well-being of life (Madusanka et al. 2015, 2016, 2017). Nanotechnology has revolutionized the medical research and has provided effective solutions due to the unique and extraordinary properties of nanoparticles. NM have been used to detect, treat, and prevent diseases including disorders in the nervous system (Siddiqi et al. 2018). As mentioned previously, one medical application of nanotechnology is currently being developed to employ NPs to deliver drugs to the target cells and tissues to cure diseases. Utilization of high-affinity ligands on the surface of the NPs increases the possibility of using them to deliver specific drug molecules to targeted cells in the nervous system (Lockman et al. 2002).

11.3 Nanocarriers

These nanoparticles have macromolecular materials which enable the drugs to be attached, adsorbed, dissolved, and encapsulated (Lockman et al. 2002). Several nanocarriers have been designed to deliver therapeutic compounds into the central nervous system (Popovic and Brundin 2006; Béduneau et al. 2007; Orive et al. 2009; Hadavi and Poot 2016; Bezerra-Sores et al. 2019). Dendrimers, micelles, nanoscale ceramics, liposomes, polymeric nanoparticles and solid lipid nanoparticles, etc., can be used as nanocarriers (Fig. 11.2) (Poovaiah et al. 2018). Quantum dots, gold nanoparticles, carbon nanotubes, and fullerenes have also been used to enhance the efficacy of drug delivery (Siddiqi et al. 2018). The drug-loaded nanoparticles are shown in Fig. 11.3 (Lockman et al. 2002).

Nanocarriers can be optimized to be delivered either through the BBB (systemic) or behind the BBB (local). For the systemic delivery, the nanomaterials have to be optimized to cross the BBB via receptor-mediated and adsorptive-mediated transcytosis pathways (Barbu et al. 2009; Yang 2010). For locally delivered nanoparticles, the particles travel behind or bypass the BBB and can be achieved through promising techniques such as convection-enhanced delivery (CED) (Debinski and Tatter 2009). CED enables therapeutic drugs to be delivered through the interstitial spaces of the central nervous system. However, several parameters still need to be investigated for a successful application of CED (Mehta et al. 2017). In both approaches, the rate of the therapeutic agent release from the nanocarrier needs to be controlled for a successful delivery (Patel et al. 2012).

It has been found that nanocarrier-mediated targeted drug delivery is more safe and efficient than conventional drug delivery systems for neurological disorders. The most interesting fact is that the therapeutic drug in the nanocarrier does not undergo any obstacles in the transport to the brain. Hence nanocarriers can be designed in such a way that it does not alter the properties of the drug (Poovaiah et al. 2018). Nanocarrier-mediated drug delivery systems exhibit slow drug release, decreasing the toxic effects (Lockman et al. 2002; Milani et al. 2019). It has been

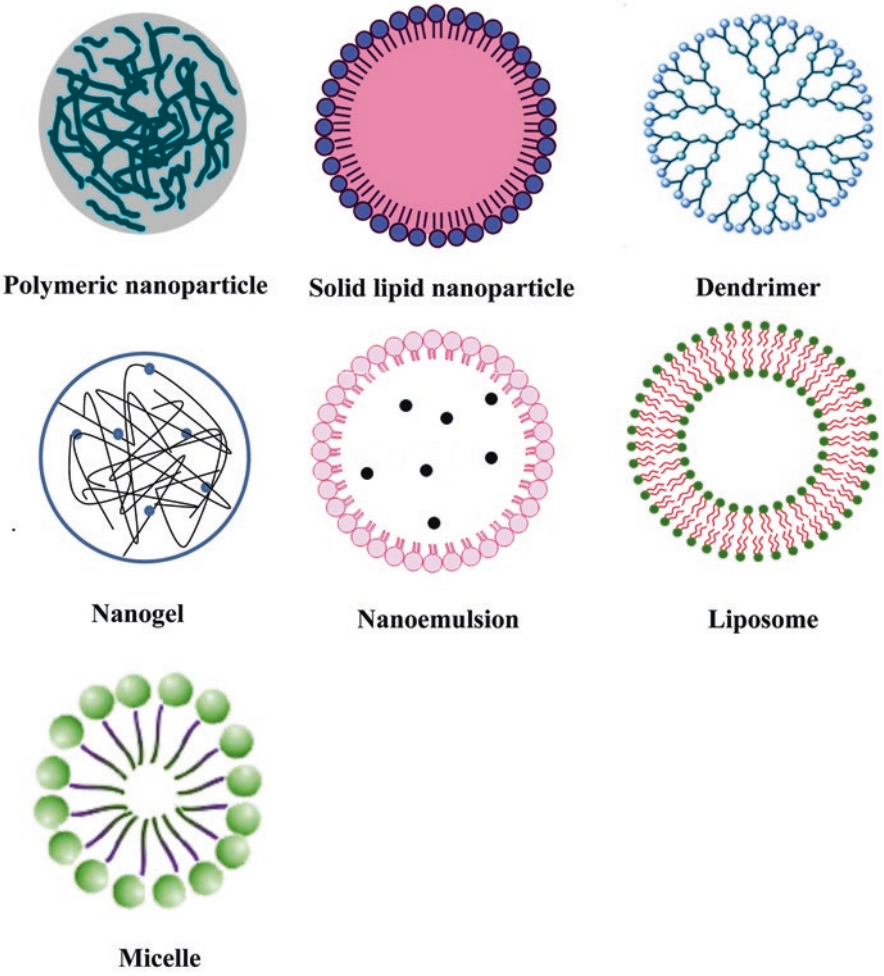

Fig. 11.2 Different types of nanoparticles that are used in the treatment of neurodegenerative diseases (Modified from Poovaiah et al. 2018)

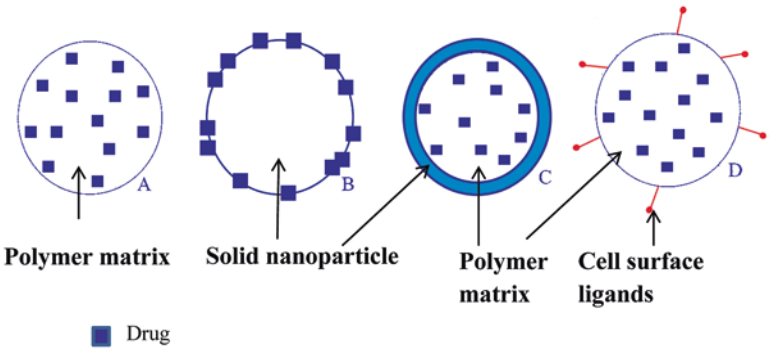

Fig. 11.3 Drug-loaded nanoparticles (Modified from Lockman et al. 2002)

suggested that the high surface area to volume ratio of NPs makes them easier to adsorb on endothelial cells with increasing the retention and permeation across the BBB. The chosen nanodrug should possess a higher level of bioavailability. NM can be designed in such a way that they will not interact with the defense mechanisms in the body. NM and polymers can be coated with surfactant polysorbate 80 to cross the BBB easily; hence they can be used in the drug delivery system. Surface of these nanomaterials can be treated with hydrophilic polyethylene glycol layer which protects the drugs from degradation by enzymes and the immune system. Therefore, NMs have additional advantages over other materials. The nanocarrier may release the drug at the site of targeted tissue by desorption, by diffusion through the NP matrix or wall of the polymer or NP erosion, or by combining the above mechanisms (Lockman et al. 2002). Overall, the advantages of using nanoparticles in drug delivery include ability to use them in targeted drug delivery by attaching ligands to the surface or using magnetic guidance, ability to load more drug, and their controlled release of drug (Spuch et al. 2012). In order to deliver drugs to the brain, the nanoparticles need to be nontoxic, biocompatible, biodegradable, stable in blood, having the ability to penetrate BBB, scalable, and cost-effective and the diameter of particles needs to be less than 100 nm (Spuch et al. 2012).

11.3.1 Transport of Nanoparticles Across the BBB

The main pathways to cross the BBB include the paracellular aqueous pathway, substrate-specific transport proteins, the transcellular lipophilic pathway, receptor-mediated transcytosis, and adsorptive-mediated transcytosis (Abbott et al. 2006; Bhaskar et al. 2010; Yang 2010; Patel et al. 2012). It has been accepted that drugs can be delivered directly to the endothelial cells in the brain using endocytosis and transcytosis (D'Souza 2019).

Lipophilic molecules with molecular weight of 400–600 Da can be diffused through the transcellular pathway down a concentration gradient. Molecules such as proteins, amino acids, and glucose need either carrier-mediated, receptor-mediated, or adsorptive-mediated transport (Cecchelli et al. 2007). A certain amount of lipophilic drugs with a molecular weight less than 500 Da can only cross the BBB due to this restrictive nature of BBB. Therefore, a vast amount of traditional drugs are unable to reach the target cells in the CNS (Pardridge 2001). In this regard, nanocarriers have gain considerable attention for the delivery of drugs overcoming the above limitations (Lockman et al. 2002; Koo et al. 2006; Barbu et al. 2009; Kreuter 2012).

Carrier-mediated transport involves the utilization of carrier-mediated proteins to transport water-soluble molecules through facilitated diffusion or active transport. These transporter proteins include GLUT1, LT1, and ABC. ABC transporters are mainly involved in the transport of drugs. But the drug molecules are removed from entering the brain by proteins such as P-glycoprotein and multiple drug resistance proteins (D'Souza 2019). Many strategies for the delivery of drugs to the brain have focused on adsorptive-mediated transcytosis (Lu 2012). However, it has been

recommended that receptor-mediated endocytosis can be used for targeted drug delivery (D'Souza 2019).

In adsorptive-mediated transcytosis, electrostatic interactions occur in the cell membrane of brain capillary endothelial cells and trigger the movement of drug molecules inside the cells (Chen and Liu 2012; Lu 2012). In receptor-mediated endocytosis, binding of ligands to the receptors triggers the internalization (D'Souza and Devarajan 2015). Hence, if nanoparticles consist of ligands, they can be transported across the membrane. As an example, the nanoparticles coated with polysorbate can bind to low-density lipoprotein receptors of CNS (Lin et al. 2017; D'Souza 2019).

It has been suggested that NP cross the BBB utilizing passive diffusion and/or receptor-mediated endocytosis. In passive diffusion, the therapeutic drug dissolves in the lipid membrane of cerebrovascular endothelial cells followed by the release of drug into the brain.

Passive diffusion is characterized by Fick's law of diffusion.

$$-\mathrm{d}C / \mathrm{d}t = k\left(C_1 - C_2\right) \tag{11.1}$$

Passive diffusion depends on the charge, molecular weight, concentration gradient, degree of protein binding, and lipophilic nature of the drug. Capillary endothelial cells consist of carrier proteins, thus enabling the transport of certain drugs across the BBB, namely carrier-mediated transport. This transport across the BBB can take place either via facilitated transport, active transport, or endocytosis.

Carrier-mediated transport is characterized by Michaelis-Menten saturation kinetics (Lockman et al. 2002).

$$\text{Rate} = \frac{V_{\max} * C + kd}{K_m + C} \tag{11.2}$$

11.3.2 Nanomaterials Aided Drug Release

Many researchers have investigated the controlled release of materials from nanoparticles/nanocomposites in various fields in nanoscience (Kottegoda et al. 2011, 2014, 2017; Madusanka et al. 2015, 2017). Pharmacokinetic analysis of the release of doxorubicin from nanoparticles is shown in Fig. 11.4. Gupta et al. (1987) represented this as a bi-exponential equation.

$$C_t = A\mathrm{e}^{-\alpha t} + B\mathrm{e}^{-\beta t} \tag{11.3}$$

Where, C_t is the concentration of drug remaining in the NP at a given time, A and B are system characteristics constants and intercepts of release, α is the initial rate constant, and β is the secondary rate constant.

According to this model, there is a rapid release of the drug from the NPs at first, possibly due to loosely bound drug to the surface of NP, followed by slower, controlled release of the drug related to either degradation of NP or diffusion of drug

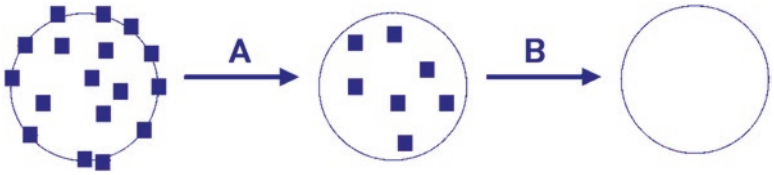

Fig. 11.4 Pharmacokinetic release of drug from the nanoparticle (Modified from Lockman et al. 2002)

via the NP shell or matrix. The final release has been analyzed by both zero-order kinetics, associated with biodegradable NPs, and first-order kinetics associated with nonbiodegradable NPs (Gupta et al. 1987; Lockman et al. 2002). The release of drug depends on the degradation of NP or erosion of nanocapsule shell and also on the structure of the NP. Hence the incorporation of the drugs inside a solid lipid NP, encapsulation in the shell of NP, adsorption onto the surface, or crosslinking to the NP affects the release of drug from the nanoparticle (Lockman et al. 2002).

11.3.3 Drug Delivery Using Colloidal Nanoparticles

Colloidal drug delivery systems include nanoparticles, dendrimers, emulsions, liposomes, etc. Particle size, surface properties, and stability of particles affect the delivery of drugs to the CNS (Bhaskar et al. 2010). As discussed earlier, ligands can be attached to nanoparticles to increase the affinity of particles.

Surfactants such as Tween 80 and poloxamer 188 can be attached to the surfaces of nanoparticles for targeted delivery of drugs (Kanwar et al. 2012). These surfactants increase the drug penetration inside the brain by inhibiting the efflux caused by the action of P-glycoprotein and have the ability to solubilize the lipids in the brain endothelial cell membrane (Kulkarni and Feng 2011). Researchers have also focused on the factors such as vascular endothelial growth factor, insulin-like growth factor, and lactoferrin for the delivery across the BBB (Farokhzad and Langer 2006).

11.3.4 Polymeric Nanoparticles (PNP)

Many polymer nanoparticles have been designed to treat neurodegenerative diseases (Fornaguera and Solans 2016). Among the different types of nanocarriers, polymer nanoparticles have more desirable properties such as being stable, high loading of therapeutic agents, control drug release, and being safe (Saltzman 2001).

Polymeric nanoparticles are ideal to be used as carriers in the drug delivery system as they have tunable characteristics. Favorable characteristics of polymeric nanoparticles are listed in Table 11.1 (Lockman et al. 2002). The solubility in water and absorption by the body is higher in PNP compared to traditional medicine.

Table 11.1 Favorable characteristics of polymeric nanoparticles (Lockman et al. 2002)

Polymer-based carriers	Polymer-based nanoparticles
• Natural or synthetic • Inexpensive • Nontoxic • Biodegradable	• Particle diameter less than 100 nm • Stable in blood (i.e., no opsonization by proteins) • BBB targeted • No aggregation of platelets • No activation of neutrophils • Noninflammatory • Prolonged circulation time • Avoidance of the reticuloendothelial system • Scalable and cost-effective

Polymer nanocarriers can be penetrated deeply into tissues and targeted cells as they are hydrophilic and their size is in the submicron range. Moreover, the efficiency and efficacy of treatment is enhanced and the preparation methods of these particles are cost-effective (Fornaguera and Solans 2016).

PNP are solid colloid particles range in size from 1 to 300 nm (Patel et al. 2012). Degradable PNP have gain more attention due to their desirable properties and their higher efficacy as nanocarriers for the treatment of neuro-related diseases. The two forms of PNP are nanocapsules and nanospheres (Patel et al. 2012; Das et al. 2016). Nanocapsules have a core shell structure whereas nanospheres consist of homogeneous matrices. Protection of the active compound, easy delivery, and permeability of drugs can be increased by tuning the nanoparticles. It has been difficult to design polymeric nanoparticles for the targeted delivery of drugs. In targeted drug delivery, the drug needed to be released only after it reaches the affected tissue or cells in neurodegenerative diseases (Tosi et al. 2013). There are only a few instances where polymer nanoparticles have become successful in targeted drug delivery (Shi et al. 2011). However, polymer nanoparticles are preferred nanocarriers as they are stable, safe, and easy to manufacture. Moreover, it shows controlled release of drugs.

11.3.4.1 Preparation Methods

Different types of polymers, stabilizers, and surfactants can be added in the preparation methods of NPs. These substances may affect the brain, drug distribution, and persistence of the drug in the blood. Therefore, care has to be taken in order to optimize the properties of nanocolloidal particles.

The main preparation methods include:

1. Emulsion polymerization
2. Interfacial polymerization
3. Desolvation evaporation
4. Solvent deposition

The therapeutic drugs can be loaded by utilizing absorption, adsorption, and encapsulation methods.

Emulsion Polymerization

Emulsion polymerization is one of the most common preparation techniques of NPs. Emulsion polymerization is more rapid than other techniques and the process does not require stabilizers and surfactants. In here, a chain of polymers are made using a given monomer unit at room temperature, initiated by either free radical or ion formation. High energy radiation, hydroxyl ions, or UV light may trigger the growth of the polymer. Polymerization is followed by filtration of the solution and removal of any residual monomers in the neutralization step. Micelles and droplets (NPs) may be resulted from polymers. The polymer provides a considerable space for the loading of the drug via absorption or adsorption (Lockman et al. 2002).

The disadvantages of this method include the requirement of free radicals, radiation, or UV light. These requirements decrease the stability of peptides and proteins that can be incorporated during polymerization. Hence, in order to increase the stability of proteins or polypeptides, purification of NPs is required via dialysis and centrifugation. These purification steps limit the scaling up of NPs. Moreover, a large amount of organic solvents are needed leading to possible toxic effects (Birrenbach and Speiser 1976; Lockman et al. 2002).

Interfacial Polymerization

Interfacial polymerization and emulsion polymerization are similar as monomer units are used to grow the polymer chain. But the mechanism used in interfacial polymerization is different to emulsion polymerization. In this method, an aqueous and organic phase is met together by homogeneous, micro-fluidization, or emulsification under mechanical stirring. As an example, production of polyalkylcyanoacrylate nanocapsules can be completed after dissolving the monomer in oil followed by slow addition to an aqueous phase using a small tube, under continuous stirring. The monomer then forms capsules in the size ranging from 200 to 300 nm by anionic polymerization. Loading of drug is achieved by adding the drug in the presence of monomer in the organic phase. Hence, the drug is incorporated inside the NP matrix (Al Khouri Fallouh et al. 1986). Another approach of interfacial polymerization can be achieved by adding a benzyl benzoate, acetone, and phospholipid solvent mixture to the drug and monomer in the organic phase.

A nanocapsule shell may be formed between the aqueous phase and the benzyl benzoate drops in the organic phase (Fessi et al. 1989). The encapsulation of drug is achieved via interfacial polymerization and the drug is protected until it reaches the site of action/target tissue. Therefore, therapeutic agent can be delivered by crossing the BBB and can be released to the target cells or tissues in the brain (Lockman et al. 2002).

Denaturation and Desolvation

Macromolecules can be used to produce NPs. These macromolecules include albumin, gelatin, etc. Oil denaturation and desolvation processes are used to process these macromolecules. In oil denaturation, large macromolecules are trapped by homogenization in an organic phase. Then, the macromolecule is slowly added to an aqueous phase under continuous stirring. The formed particles after introducing two immiscible phases can be strengthened by aldehyde crosslinking (Tomlinson and Burger 1985) or denaturation by heat (Zolle et al. 1970). The resultant particle size largely depends on the type of oil used rather than quantity of the macromolecules, emulsification time, and temperature used in the process (Lockman et al. 2002).

Desolvation process can also be used to form NPs by macromolecules. In here, the macromolecule is dissolved in a solvent and the macromolecule becomes swollen and coiled in the solvent. Changing the pH, charge, and environment or using ethanol as a desolvating agent induces the swollen macromolecule to be coiled tightly. The drug that is bound to macromolecule becomes trapped in the formed particle. The macromolecule can then be strengthened by aldehyde crosslinking. The major disadvantage of this method is the production of lower quantity of NPs and the drug compared to previous methods (Lockman et al. 2002). Solid lipid nanoparticles are manufactured by homogenization under high pressure. The solid matrix facilitates a controlled release of drug rather than the sudden release of drug observed in fat emulsions (Lockman et al. 2002).

11.3.5 Solid Lipid Nanoparticles (SLNPs)

SLNPs serve as a potential candidate to serve as an efficient nanocarriers for drug delivery in neurodegenerative diseases. The release and stability of drugs are higher in solid lipid nanoparticles; hence, it minimizes the cytotoxic effects in the tissue (Roney et al. 2005). Solid lipid nanoparticles exhibit more stability and better shield against enzymes in the body compared to colloidal delivery systems. Also, the rejection of the drug by the CNS is minimal after encapsulating inside a matrix. Therefore, solid lipid nanoparticles (SLNPs) have more potential to treat neurodegenerative diseases (Yoo et al. 2005).

In solid lipid nanoparticles, the drug is either dissolved or distributed in the matrix. It is consisting of a solid lipid core which is hydrophobic. Fenart et al. (1999) investigated the effect of lipid coating of nanoparticles and their movement across a blood-brain barrier consisting of bovine brain capillary endothelial cells and rat astrocytes (in vitro BBB). They have used neutral, anionic, and cationic nanoparticles for the investigation. To make the nanoparticles charged, NPs have crosslinked with phosphate (anionic) and quaternary ammonium (cationic) ligands. Lipid-coated ionized nanoparticles have crossed the BBB having a threefold to

fourfold increase comparatively to non-coated particles. It has been shown that lipid-coated nanoparticles penetrate the BBB by transcytosis without undergoing any alterations and the transport of nanoparticles has also not changed the integrity of the BBB. They have also loaded albumin in the cationic lipid-coated particles and the transport has shown 27-fold increase.

SLNPs have advantages over polymeric nanoparticles such as they do not use organic solvents and toxic monomers and they can be scaled up easily. Moreover, the contact time with BBB and the transport efficiency is higher for SLNPs. The SLNPs can be made biodegradable and biocompatible making them safer to use in drug delivery systems (Kaur et al. 2008). SLNPs have also demonstrated increased ability in controlling the release of drug (D'Souza 2019). However, hydrophobic SLNPs can stimulate interactions with opsonins (plasma proteins). Also, the negatively charged surfaces activate the complement system; thereby phagocytosis by macrophage takes place (Aggarwal et al. 2009). Therefore, the retention in the systemic circulation is low for SLNPs (Moghimi et al. 1991). To prevent phagocytosis, the hydrophilic surfactants of polymers can be attached to the nanoparticle surface (D'Souza 2019). SLNPs also show poor loading of hydrophilic drugs (D'Souza 2019).

11.3.6 Silica NPs

Silica NPs are more popular in the research field owing to their unique properties (Singh et al. 2014; Suriyaprabha et al. 2014; Shirshahi and Soltani 2015). Mesoporous silica NPs (MSNPs) with 2–50 nm pore size are potential candidates in nanomedicine to be used in controlled and targeted drug delivery (Vivero-Escoto et al. 2010; Bharti et al. 2015; Giret et al. 2015; Martínez et al. 2015). MSNPs have high surface area to volume ratio and a pore; hence more drug molecules can be loaded (Zhang et al. 2010; Douroumis et al. 2012). The pore size can be controlled; thus, it has better drug loading and release (Silva Adaya et al. 2017). The surface can be modified for targeted drug delivery leading to efficient drug delivery and reduced toxicity. Moreover, silica NPs are biodegradable and exhibit reduced cytotoxicity in living organisms. Silica NPs when combined with magnetic and luminescent materials can be used to bio-image and deliver drugs simultaneously (Li et al. 2013; Wang and Gu 2015).

11.3.7 Dendrimers

Dendrimers are branched nanosized molecules. Also, they are monodispersed and homogeneous (Klajnert and Bryszewska 2001; Abbasi et al. 2014). They are excellent candidates to be in controlled targeted drug delivery. They are also open to

many functional groups and their interior and exterior surfaces are hydrophilic. Dendrimers as nanocarriers are a good solution for the drugs which show poor solubility (Silva Adaya et al. 2017).

11.3.8 Nanocomposite Hydrogels (NC Hydrogels) and Nanogels

In NC hydrogels nanoparticles have been added to a hydrogel matrix (Schexnailder and Schmidt 2009; Satarkar et al. 2010). Swelling ratio, diffusion coefficient, and mesh size of NC gels may affect the release of drug from the matrix of the hydrogel. These properties can be altered to increase the efficiency of drug delivery. Moreover, they have excellent biocompatibility and biodegradability (Song et al. 2015). Therefore, NC hydrogels have the potential to become an excellent nanocarrier in drug delivery.

Crosslinked network of polymers as hydrophilic nanoparticles (nanogel) has gained attention in therapeutic drug delivery (Vinogradov et al. 2005). Researchers have studied a nanogel consisting of crosslinked polyethylenimine (PEI) network and poly(ethylene glycol) (PEG) molecules, as a nanocarrier (Vinogradov et al. 1999; Lemieux et al. 2000). It has shown promising results in drug delivery due to its increased swelling capacity and low buoyant density. Also, the polymer has the ability to protect the loaded biodegradable drug (Vinogradov et al. 2005).

Oligonucleotides have suggested as a potential therapeutic agent for neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease (Boado et al. 1998; Vinogradov et al. 2004; Seidman et al. 2011). However, oligonucleotides are more prone to degradation by enzymes and removal through excretion; thus, it shows poor stability in the body. Also, BBB does not allow oligonucleotides to pass through (Vinogradov et al. 2004). The ultimate solution for this issue is encapsulating oligonucleotides in nanoparticles to be delivered to the brain (Kreuter 2012). In this case, nanogels are preferred over currently used nanoparticles owing to their unique properties (Vinogradov et al. 1999, 2002). According to Vinogradov et al. (2004), a nanogel containing oligonucleotides has crossed the BBB in vivo, while the liver and spleen have shown poor uptake of the nanogel.

11.3.9 Nanoemulsions

Nanoemulsions consist of oil, water, and an emulsifier. A surfactant can be used as the emulsifier. They can be prepared using either high energy or low energy methods. Nanoemulsions are attractive materials to be used in drug delivery owing to their small size, increased stability, and tunable rheology (Gupta et al. 2016). Highly lipophilic drugs which are water insoluble can be transported to the brain using nanoemulsions (Wong et al. 2010).

11.3.10 Liposomes

Liposomes are widely used as nanocarriers (Webb et al. 2008). They are small vesicles containing phospholipid bilayers. Liposomes have shown promising results due to their small size, biocompatibility, and the ability to deliver both lipophilic and hydrophilic drugs (Spuch and Navarro 2011).

Calcitonin is a hydrophilic drug having a higher molecular weight. The BBB prevents the entry of such molecules. Researchers have investigated the uptake of calcitonin in liposome nanocarriers by the brain, in vivo, and the liposome containing the drug could pass the BBB (Chen and Lee 1993). Also, cationic liposomes have been used to encapsulate the genetic drugs. Therefore, the genetic drug can be carried to the target site in the brain by crossing the BBB. They take the shape of a hexagonal structure (lipoplexes) due to the electrostatic attractions between positively charged liposomes and the negatively charged genetic drug (Poovaiah et al. 2018).

11.3.11 Micelles

Amphiphilic molecules have a hydrophilic head and a hydrophobic tail, enabling them to form nanosized structures known as micelles. Hydrophobic drugs can be loaded at the core of the micelle to deliver drugs (Poovaiah et al. 2018). Pluronic block copolymer P85 has exhibited the ability to penetrate the BBB by inhibiting the P-glycoprotein drug efflux system. In aqueous solutions, amphiphilic pluronic block copolymers are capable of self-assembling into micelles above critical micelle concentration. P85 micelles have shown an enhanced permeation of drugs through an in vitro model of BBB, which is a monolayer of bovine brain microvessel endothelial cells (Batrakova 2003).

11.4 Nanocarriers in Neurodegenerative Diseases

11.4.1 Alzheimer's Disease

Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline), a derivative of quinolone, can be used to treat Alzheimer's disease. This clioquinol can be loaded in n-butyl-cyanoacrylate nanoparticles to be delivered across the BBB (Roney et al. 2005). Donepezil is also used as a therapeutic drug in Alzheimer's disease. Poly(lactide-co-glycolide) (PLGA) nanoparticles can successfully carry donepezil to the brain (Mathew et al. 2012). At first, it has shown burst release followed by a slow release at the site of action (Md et al. 2014).

11.4.2 Parkinson's Disease

Polymeric nanoparticles have drawn attention in the drug delivery for the treatment of Parkinson's disease. Polysorbate 80-coated poly(butylcyanoacrylate) (PBCA) nanoparticles could deliver drugs to the brain by crossing the BBB (Olivier 2005). Polysorbate 80 facilitates the transport through the BBB by interacting with the BBB (Patel et al. 2012).

One of the main reasons behind Parkinson's disease is the decrease of dopamine levels. Nanoparticles made from chitosan which is a naturally occurring polymer can also be used to deliver peptides and dopamine to the brain. Hence chitosan nanoparticles are promising candidates for the treatment of Parkinson's disease (Patel et al. 2012; Songjiang and Lixiang 2009; Trapani et al. 2011). Also, curcumin as a therapeutic drug has shown a possibility to treat disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD) (Darvesh et al. 2012).

Another potential treatment strategy is the delivery of genes with the help of nanocarriers to the CNS. These genes can be used to improve the regeneration and compensatory mechanisms of the brain (Linazasoro 2008).

11.4.3 Huntington's Disease

Fullerenols, derivatives of fullerenes, serve as an ideal nanocarrier owing to their spherical and hollow structure for the treatment of Huntington's disease. The major reason behind using fullerenols is the antioxidant nature of them. The hydrophilic fullerenols have the capability to remove free radicals (Grebowski et al. 2013) exhibiting a great potential as an therapeutic approach for Huntington's disease (Jin et al. 2000; Poovaiah et al. 2018). SLNPs have also investigated for the treatment of Huntington's disease. Nitrendipine can be used as a therapeutic drug in Huntington's disease. However, the hydrophilic nature of this drug restricts its movement across the BBB. Studies have shown that uptake of nitrendipine by the brain increases when encapsulated in SLNPs made of different types of glycerides compared to the nitrendipine alone (Manjunath and Venkateswarlu 2006). Researches have also used modified cyclodextrin to encapsulate short-interfering RNA (siRNA) to suppress the expression of mutant huntingtin (HTT) gene. The cytotoxic effects have also found to be low in this method (Polt et al. 1994; Godinho et al. 2013).

11.5 Conclusion and Future Prospects

Targeted drug delivery exhibits better efficacy and efficiency and is preferred over traditional therapies. At present, many research groups are progressively working on the designing of nanomaterials for targeted drug delivery in neurodegenerative

diseases (Guzman-Villanueva et al. 2019). Nanocarrier-mediated targeted drug delivery strategies are still in the experimental stage and they may become ideal for the treatment of neurodegenerative diseases in the near future. Even though there are many popular and accepted theories for the transport of nanomaterials across the brain, the exact mechanism of uptake is not yet discovered. Therefore, further research need to be carried out to discover the above mechanisms. Nanoparticles should be designed in such a way that they are biodegradable, biocompatible, and nontoxic for a successful delivery of drugs. Also, more research is required to increase the loading efficiency of certain drugs. Use of nonbiodegradable nanoparticles leads to the accumulation of them in the brain leading to toxic side effects. The fate of such particles and their side effects are still unknown. Therefore, more investigations are needed to validate the use of nanocarriers for the treatment of neurodegenerative diseases.

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Chapter 12
Gold Nanoparticles in Diagnosis and Treatment of Alzheimer's Disease

Senthilkumar Sivanesan and Shanmugam Rajeshkumar

Abstract Gold nanoparticles are one of the most important nanoparticles with many applications in diagnosis such as tumor markers detection, microbial pathogen detection, and many imaging techniques and therapies like gene therapy, cancer therapy, and drug delivery systems. Apart from this, these are also used in information technology and many electronic and communication technologies. Delivering of drug across the blood brain is a major problem in the neurodegenerative diseases majorly Parkinson and Alzheimer's diseases. The gold nanoparticles are golden opportunities in the treatment of neurodegenerative diseases and drug delivery in the blood-brain barriers and inducing the neuronal activities. This chapter clearly explains about the gold nanoparticles and its very clear applications in the blood-brain barrier hurdle to treat neurodegenerative diseases, biomarkers for Alzheimer's disease, diagnostics targeting, and inhibiting amyloid fibrils in AD and role of gold nanoparticles in the Alzheimer's disease treatment.

Keywords Gold nanoparticles · Characterization · Alzheimer's disease · Neuron · Neurodegenerative disease · Treatment

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12.1 Introduction

12.1.1 Nanotechnolgy and Nanoparticles

Nanotechnology is one of the most promising and new areas of research in modern science. Nanoparticles possess new and improved properties of material which are mainly based on size, shape, distribution, and morphology than large particles from which the nanoparticles are made (Rajeshkumar and Bharath 2017). Nanoparticles have a higher surface area which gives larger target interaction. It has many important properties such as low melting point, catalytic activity, high photoconductivity, and high semi-conductivity (Santhoshkumar et al. 2017). For the synthesis of metallic nanoparticles, living extracts have been utilized by researchers. They followed easy processes such as the procedures of reducing the metal ions. In doing so, they made use of biomass extracts as a basis of extracellular or intracellular reductants (Rajeshkumar and Poonam 2018).

Gold nanoparticles are playing a major role in the field of biomedicine. Green synthesis of gold nanoparticles is an emerging field. This includes different sources such as microorganisms (bacteria, fungi, and yeast) and plant materials for synthesis of nanoparticles. The bioactive compounds of plants and microorganisms used for the biosynthesis of gold nanoparticles are enlisted (Table 12.1 and Fig. 12.1).

Alzheimer's disease (AD) is one of the age-related neurodegenerative diseases affecting several millions of people globally. The deposition of β-amyloid peptide (Aβ) fibrils in a form of extracellular plaques and intracellular accumulation of tangles are key pathological signs of AD. With the aging population, there is proportionate increase in the incidental rate of neurodegenerative disorders in recent years. It is expected in future years that the disease may increase by roughly 30% among the aged population. For AD diagnostic approaches, blood is the most preferred parameter than CSF as it contains various kinds of biomarkers such as amyloid beta 1–40, 1–42, and τ (tau) protein (Kim et al. 2019). With the present technology, we lack accuracy and hence we end up with low-level detection of AD biomarkers with blood plasma-based methods owing to low concentration of AD biomarkers in blood plasma and other interfering blood factors that reduce the accuracy and precision.

12.2 Characterization of Nanoparticles

The synthesized gold nanoparticles are characterized by using various techniques such as UV-vis spectrophotometry (surface plasmon resonance), scanning electron microscopy, transmission electron microscopy (morphology size and shape), X-ray diffraction (cubic structure and crystalline nature), Fourier transform infrared spectroscopy (bioactive compounds responsible for nanoparticles synthesis), and elemental dispersive analysis (elements present in the prepared nanoparticles). The different characterization techniques used for nanoparticles confirmation are shown in Fig. 12.2.

Table 12.1 Green synthesis of gold nanoparticles

S. no	Name of the reducing agent	Size	Shape	UV-vis/FTIR	Applications	Reference
1	*Deinococcus radiodurans* (tetrachloroauric acid)	43.75 nm (DLS)	Spherical, triangular, irregular shapes	540 nm OH or NH groups of carbohydrates and proteins	Antibacterial activity against *E. coli* and *Staphylococcus aureus*	Li et al. (2016)
2	*Enterococcus* sp. (chloroauric acid) (extracellular)	6–13 nm (TEM)	Spherical shape	545 nm	Anticancer activity against lung and liver cancer cells	Rajeshkumar (2016)
3	*Staphylococcus epidermis* (tetrachloroaurate) (extracellular)	20–25 nm (TEM)		545–550 nm C=C or C-H	Catalytic activity for methylene blue degradation	Srinath and Rai (2015)
4	*Rhodopseudomonas capsulata* (tetrachloroauric acid) (extracellular)	10–20 nm (TEM)	Spherical shape	530 nm N-H group		He et al. (2008)
5	*Pseudomonas aeruginosa*, *Rhodopseudomonas capsulata* (hydrogen tetrachloroaurate) (extracellular)	10–20 nm (TEM)	Spherical shape	540 nm		Singh and Kundu (2013)
6	*Bacillus marisflavi* (hydrogen tetrachloroaurate) (extracellular)	14 nm	Spherical	560 nm	Catalytic reduction of Congo red and methylene blue	Nadaf and Kanase (2016)
7	*Bhargavaea indica* (gold chloride trihydrate) (extracellular)	106 nm	Flower-shaped nanoparticle (FE-TEM)	536 nm	F-shaped gold nanoparticles may have the potential for multifunctional use, when compared to spherical nanoparticles, for medical applications such as targeting, diagnosis, photo imaging, and drug delivery	Singh et al. (2016)
8	*Bacillus* sp. (auric chloride)	20–50 nm (SEM)	Spherical shape	540 nm		Biradar and Lingappa (2012)
9	*E. coli* DH5α (chloroauric acid)	20–30 nm (TEM)	Spherical and few triangles	535 nm	The NPs are bound to the surface of the bacteria and this composite may be used for application in realizing the direct electrochemistry of Hb	Du et al. (2007)

(continued)

Table 12.1 (continued)

S. no	Name of the reducing agent	Size	Shape	UV-vis/FTIR	Applications	Reference
10	*Acinetobacter* ($HAuCl_4$)	Lowest cell density (~19 nm) Highest salt concentration (~39 nm)	Spherical Polyhedral, triangular, spherical (TEM)	540 nm FTIR-amide I and II groups	Cell density and gold chloride concentration have tremendous effect on synthesis and morphology of AuNP	Wadhwani et al. (2018)
11	*Pseudomonas aeruginosa* (hydrogen tetrachloroaurate) (extracellular)	(TEM) *Pseudomonas aeruginosa* ATCC 90271 (30 ± 10) *Pseudomonas aeruginosa* 2 (25 ± 15) *Pseudomonas aeruginosa* 1 (15 ± 5)		543 nm 540 nm 531 nm		Husseiny et al. (2007)
12	*Shewanella algae* ($HAucl_4$)	9.6 nm (nanoparticle) 100–200 nm (nanoplate)	Spherical	Carbonyl group		Konishi et al. (2010)
13	*Bacillus cereus* and *Fusarium oxysporum* ($HAuCl_4$)	20–50 nm	Spherical, hexagonal, and octagonal (TEM)	510–530 nm	Toxic effect in the human fibroblast cell line CIRC-HLF	Pourali et al. (2017)
14	*Bacillus marisflavi* ($HAuCl_4$)	14 nm	Crystalline and spherical (TEM)	486 nm	Catalytic dye degradation of Congo red and methylene blue (GC-MS)	Nadaf and Kanase (2016)
15	*E. coli* and *Desulfovibrio desulfuricans* ($HAuCl_4$)	5–50 nm (TEM)	Nanospheres, nanorods, or triangular nanoprisms	~545 nm		Deplanche and Macaskie (2008)

16	*Geobacillus* ($HAuCl_4$) (intracellular)	10–20 nm		540 nm	Crude extracts of the microorganism could catalyze the NADH-dependent Au3+ reduction	Correa-Llanten et al. (2016)
17	*Pseudomonas fluorescens* (extracellular)	50–70 nm	Nanocrystalline gold	540 nm C-H		Radhika and Suman (2012)
18	*Shewanella algae* ($HAuCl_4$) (extracellular)	10–20 nm			Intracellular recovery of gold using *Shewanella algae*	Konishi et al. (2006)
19	*Trichoderma harzianum* (chloroauric acid) (extracellular)	38 (DLS) 26–34 (TEM)	Spherical (TEM)	370 nm	–	Tripathi et al. (2014)
20	*Alternaria alternate* (extracellular)	2–30	Spherical, hexagonal, and triangular (AFM)	540 nm	–	Sarkar et al. (2012)
21	*Aureobasidium pullulans* (intracellular)	29 ± 6	Spherical	No absorbance	–	Xiao-rong et al. (2011)
22	*Fusarium* sp. (intracellular)	125 ± 83	Spherical	No absorbance	–	Xiao-rong et al. (2011)
23	*F. oxysporum* (intracellular)	128 ± 70	Spherical	280 nm	–	Xiao-rong et al. (2011)
24	*Phanerochaete chrysosporium* (extracellular and intracellular)	10–100 (AFM)	Spherical	280 nm	–	Sanghi (2011)
25	*Colletotrichum* sp.	8–40 (TEM)	Spherical	551 nm	–	Shiv Shankar et al. (2003)
26	*F. solani* (chloroauric acid) (extracellular)	41 (AFM) 20–50 (TEM)	Spherical (TEM)	527 nm	–	Gopinath and Arumugam (2014)
27	*A. niger* ($HAuCl_4$) (extracellular)	10–30 (TEM, SEM)			Larvicidal activity against *A. stephensi*, *C. quinquefasciatus*, and *A. aegypti*	Soni and Prakash (2012)

(continued)

Table 12.1 (continued)

S. no	Name of the reducing agent	Size	Shape	UV-vis/FTIR	Applications	Reference
28	*C. albicans* ($HAuCl_4$) (extracellular)	60–80 (low conc.) 20–40 high conc. (TEM)	Hexagonal, triangular, and spherical (TEM)	540 nm	Interaction with tumor cells	Chauhan et al. (2011)
29	*F. oxysporum* aqueous $AuCl_4$ (extracellular)	20–40 (TEM)	Spherical and triangular (TEM)	545 nm		Mukherjee et al. (2001)
30	*Trichothecium* sp. ($HAuCl_4$) (extracellular and intracellular)	5–200 (TEM) 10–25 (TEM)	Polygons (mainly triangles and hexagons) Hexagonal and triangular	535 nm 540 nm		Ahmad et al. (2005)
31	*V. luteoalbum* (extracellular)	10	Triangles, hexagons, spheres, and rods			Gericke and Pinches (2006)
32	*Bacopa monnieri* (*BLE*) (Valaarai)	3–45 (TEM)	Spherical	3–45 nm	Anticancer activity	Babu et al. (2013)
33	*Terminalia catappa*	10–35	Spherical	524 nm O-H group of phenols	–	Ankamwar (2010)
34	*Dracocephalum kotschyi*	11	Spherical	536 nm	Anticholinesterase agent	Dorosti and Jamshidi (2016)
35	*Olea europaea*	50–100	Triangular, hexagonal, and spherical	530–545 nm presence of proteins	Antioxidant activity	Khalil et al. (2012)
36	*Mangifera indica*	~20	Spherical	Flavonoids, terpenoids, and thiamine	–	Philip (2010)
37	*Cacumen Platycladi*	2–70	Spherical and triangular	–		Wu et al. (2013)

38	*Abutilon indicum*	1–20	Spherical	535 nm	Anticancer HT-29 colon activity	Mata et al. (2016)
39	*Butea monosperma*	20–80	Large spherical	O-H stretching polyphenol	Cancer therapeutics	Patra et al. (2015)
40	*Suaeda monoica*	14.5	Spherical and rarely triangular	535 nm flavonoids, terpenoids, and soluble proteins	Antioxidant activity	Arockiya et al. (2015)
41	*Ipomoea carnea*	25–100	Triangular, hexagonal, pentagonal, rod, and truncated	C–N stretch of amines	–	Abbasi et al. (2015)
42	*Geranium* sp.	12 ± 3	–	Carbonyl stretching	–	Franco-Romano et al. (2014)
43	*Phoenix dactylifera*	32 and 45	Spherical	544 and 538 nm -OH and -C=O groups	Catalytic activity	Zayed and Eisa (2014)
44	*Sesbania grandiflora*	34.11	Spherical	534 nm Flavonoids and polyphenols	Dye degradation	Das and Velusamy (2014)

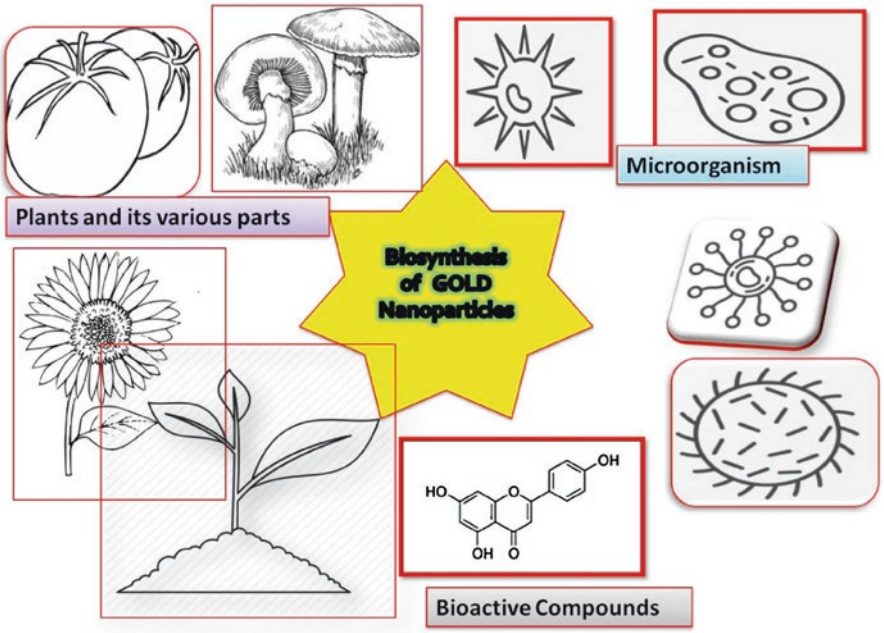

Fig. 12.1 Biosynthesis of gold nanoparticles

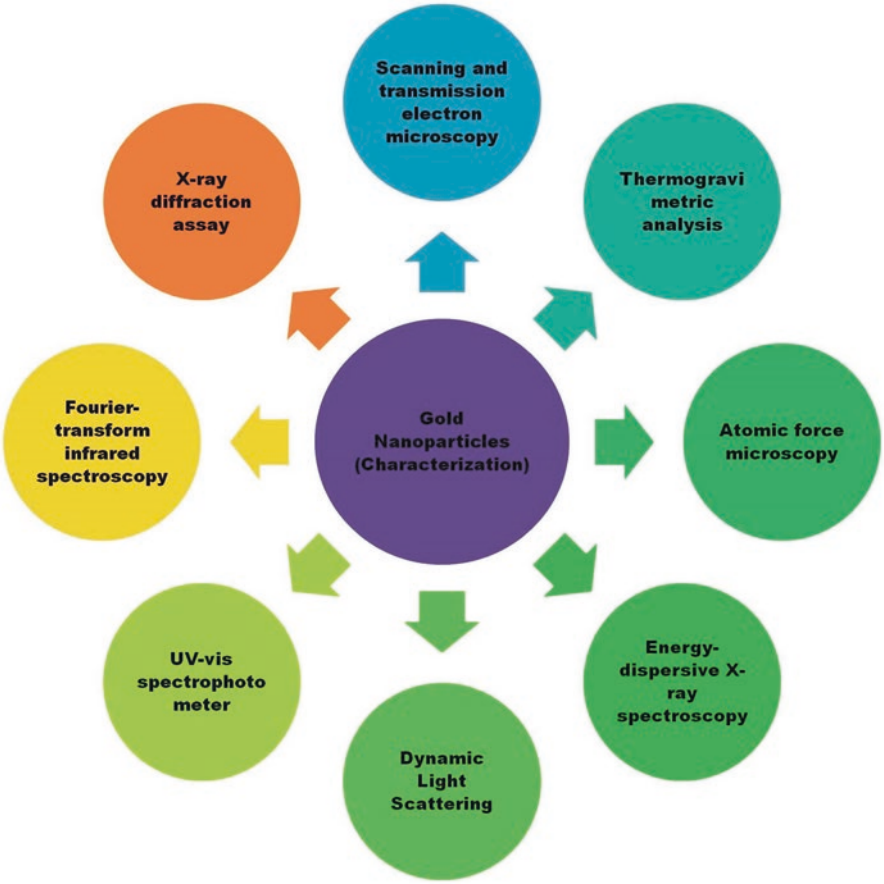

Fig. 12.2 Different characterization used for nanoparticles

12.3 Alzheimer's Disease

Despite continuous efforts in screening and testing numerous drug candidates against AD, agents such as acetylcholinesterase inhibitors are currently utilized for only symptomatic relief as clinical therapy. Several limitations are ahead of researchers in achieving targeted drug delivery of drugs to the central nervous system (CNS). The hindering factors are, for example, meagre solubility, low bioavailability, and reduced efficiency due to the impediments of the blood-brain barrier (BBB). Recent advances in nanotechnology are promising that can help to overcome such limitations in delivering drug candidates. However, current focus on nanodrug delivery systems has increased the knowledge and applications of targeted drug delivery of several therapeutic moieties. In fact, there is improved penetration of drug molecules across the CNS in order to achieve better bioavailability. A wide range of nano-carriers, such as polymers, emulsions, lipo-carriers, carbon nanotubes, metal-based carriers, etc., have been employed and studied extensively to develop successful therapeutics with sustained release and improved efficacy (Siddiqi et al. 2018). In this chapter, we try to focus on the advancements and success reported in the nano-gold-based drug delivery for treatment of AD. In recent years, the nanoparticles (NP) have shown their application and importance in diagnostics as well as therapeutics of AD. The most commonly used NPs in the field of AD research for diagnostic and therapeutic applications involve polymeric NPs (PPs), gold NPs, gadolinium NPs, selenium NPs, protein-based NPs, polysaccharide-based NPs, etc. The vast application of AuNPs in biomedical field in the present scenario could be sensed and includes controlled drug delivery; photodynamic therapy, i.e., cancer treatment; amyloid fibril inhibition; biomedical imaging; diagnosis; transplacental treatment; and for the development of specific scaffolds (Cabuzu et al. 2015).

12.4 Nanoparticles in the Edge of Overcoming Blood-Brain Barrier Hurdle to Treat Neurodegenerative Diseases

The specific transport of drugs/molecules into the brain vasculature is challenging and if made successful it may in turn facilitate the drug transport processes through the BBB especially in brain ischemic and neurodegenerative disorders. The neural tissue and circulating blood are connected in between by the blood-brain barrier (BBB) system. The key role of BBB is to control brain homeostasis as well as ion and molecule movement. Any failure or abruptness in maintaining any of these components results in the breakdown of this specialized multicellular structure that leads to neuroinflammation and neurodegeneration. The BBB is impaired in several pathological conditions such as stroke and Alzheimer's (AD) and Parkinson's disease (PD).

Small molecule inhibitors/drug candidates designed for AD treatment unfortunately cannot pass through the brain-blood barrier (BBB). To circumvent this problem, researches are trying various nanoparticles (NPs) or nanomedicines (NMs) for efficient drug delivery to CNS (Gupta et al. 2019). Interesting studies have shown

that the NPs hold the potential to initiate the receptor as well as adsorptive-mediated transcytosis that indeed help the transcellular transport of nanoparticles from the blood to the brain (Gupta et al. 2019; Lu 2012). Ruff et al. (2017) using an in vitro model of blood-brain barrier revealed that small hollow gold nanospheres/nanorods (1.4 nm) hold the potentials for improved BBB integrity of ß-amyloid-specific peptides. Using Balb/C mice, it was shown that insulin-coated gold nanoparticles (INS-GNPs) of size (20, 50, and 70 nm) can efficiently cross BBB and could be seen (2 h postinjection) in several brain regions which are associated with neurodegenerative conditions through in vivo computed tomography (CT) imaging (Betzer et al. 2017).

12.5 Engineered Gold Nanoparticles Used as Biomarkers for AD Diagnostics

Several nanobiosensor-based diagnostic tools have been designed and tested as biomarkers for AD diagnostics. Some of them employed for AD diagnostics are discussed below: The porous magnetic microspheres (PMMs) are efficient capturing/pre-concentrating platform used for detection of Alzheimer's disease (AD) biomarkers (de la Escosura-Muñiz et al. 2015). These PMMs help immobilization of antibodies on the particle surface, thereby promoting an enhanced efficiency to capture analytes/AD biomarkers efficiently from human serum samples. The catalytic activity of the AuNPs was enhanced owing to high functionality PMMs after interaction and the electrocatalytic tags thus obtained are then used for efficient analyte detection. They are used for detecting beta-amyloid and ApoE in AD-suffering patient samples such as cerebrospinal fluid (CSF), serum, and plasma. Another platform designated for nanoplasmonic biosensor employs gold nanorods and guanidine hydrochloride as a chaotropic agent for precise detection of Alzheimer's disease biomarkers in human plasma samples (Kim et al. 2019). Guanidine hydrochloride helps to overcome the impediment associated with blood-based AD diagnostics. This technique applies a sensitive aspect involving a localized surface plasmon resonance (LSPR) which holds an added advantage wherein the blood biomarker tau protein could be analyzed in disease condition even from mild cognitive impairment.

Brain amyloid-β oligomers, the probable key pathological entities/causative species of AD, are also effectively probed using gold nanoparticles (Elbassal et al. 2017). Based on the data obtained from the intensity of the surface plasmon resonance (SPR) absorption band of the AuNPs, Aβ40 amyloid oligomers/fibrils are detected and semi-quantified and effectively probed using this method. This kind of approach could be helpful for detecting early protein self-assembly and fibrillogenesis in disease condition. Tau protein, which is considered as an important biomarker of AD, is easily detected in cerebrospinal fluid based on another approach employing gold nanoparticle-based immuno-PCR (Stegurová et al. 2014). It involved the application of gold nanoparticles with tau-specific monoclonal antibody and oligonucleotide template through immuno-polymerase chain reaction (Nano-iPCR) for tau quantification. This platform was found to be more robust than conventional ELISA method used for tau protein detection. Another simple and ultrasensitive sandwich

assay for the detection of tau protein was developed (Zengin et al. 2013) by combining monoclonal anti-tau functionalized hybrid magnetic nanoparticles and polyclonal anti-tau immobilized gold nanoparticles. Here, the magnetic silica particles coated with poly(2-hydroxyethyl methacrylate) via surface-mediated reversible addition-fragmentation chain transfer (RAFT) polymerization were later biofunctionalized with monoclonal anti-tau that would specifically collect tau using magnetic approach. After the tau gets separated from the sample matrix, it was sandwiched with the surface-enhanced Raman scattering (SERS) substrate which comprises of polyclonal anti-tau and 5,5-dithiobis(2-dinitrobenzoic acid) on gold nanoparticles. The limit of detection was even as low as 25 fM (femto Moles).

Having seen that miR-137 could be one of the potential blood-based biomarkers for Alzheimer's disease diagnosis, another simple and rapid nanobiosensor approach tool such as gold nanoparticle DNA-based nanobiosensor has shown advantage for detection of microRNA associated with AD (Delkhahi et al. 2017). Chew et al. (2012) explored that miRNAs in blood could be useful as biomarkers for disease diagnosis. Based on the miRNA microarray analysis data, it was shown that after intravenous gold nanoparticle (AuNP) administration, miR-298 was found to be increased at 1 week postinjection. The work emphasized that blood miRNAs could be valuable as biomarkers after exposure with nanoparticles (Chew et al. 2012). It is important to notice that miR-298 is involved in the regulation of β-amyloid (Aβ) precursor protein-converting enzyme-1 (BACE1) in AD.

Another sensitive approach utilizes the genomic DNA samples wherein enzyme-assisted electrochemical detection enables signal amplification by using ferrocene (Fc)-capped gold nanoparticles modified with streptavidin (Lu et al. 2018). Using this method, the apoE4 gene in genomic DNAs was detected at less than 0.1 pM (picomoles) level. Another ultrasensitive electrochemical apatasensor method for early AD diagnosis was reported recently (Negahdary and Heli 2019). This approach revealed immobilization of a specific RNA aptamer on the gold nanostructure (synthesized by electrodeposition using polyethylene glycol) that helped binding of Aβ peptide and further detection by ferro-/ferricyanide redox marker. The Aβ was detected in a linear range of 0.002–1.28 ng mL^{-1}. Another development in the field is a fluorescence-based sensor array which applies gold nanoclusters for discriminating between multiple proteins at nanomolar concentrations present in sera or urine (Han et al. 2019). Advancement in the application of gold nanoparticles helps to correlate the relationship between cell cycle phases and β-amyloid peptide expression levels which could be valuable for better understanding of AD neuropathogenesis and subsequent development of therapeutics (Wang and Wang 2015).

12.6 Gold Nanoparticles Employed for Targeting and Inhibiting Amyloid Fibrils in AD

The aggregation of peptides into amyloid fibrils is one of the key events seen in the development of several neurodegenerative diseases, such as Alzheimer's disease. The various forms of AuNPs are widely used to explore the inhibition of amyloid

aggregation and mechanisms reinforced. Several possible mechanisms have been explained on the amyloid peptide nucleation and aggregation process in the presence of nanoparticles. However, it is important to realize that several contributing factors are involved in deciphering the interplay of NPs and aggregating amyloid peptides. The surface properties of the nanoparticles, the nature/chemistry of the peptides, the interacting force between the nanoparticles, and the peptides are indeed crucial to decide whether amyloid peptide aggregation is inhibited in the presence of nanoparticles. In fact a recent study helped to observe the behavior of amyloid beta peptide at phospholipid membrane buildup on gold nanoparticles with a diameter of 100 nm (Suga et al. 2018). By using membrane surface-enhanced Raman spectroscopy (MSERS) method, the enhanced signals that were detected after combining Aβ with PL (phospholipid)-layered gold nanoparticles helped to study the aggregation propensity of amyloid peptide clearly. The capacity of nanoparticles (NPs) to work even at low substoichiometric ratios apart from other convincing properties such as amendable size, shape, and surface creates an efficient strategy to be used with rationally designed Aβ aggregation inhibitors.

Engineered nanoparticles (ENPs) exhibit different roles on peptide fibrillation and could lead to deleterious biological consequences upon direct contact with human biological system. Therefore handling of such materials should ensure safety in preparation as well as method of delivery. The potentials of gold nanoparticles (AuNPs) such as gold nanospheres (diameter ∼20 nm) and gold nanocubes (edge length ∼20 nm) of various shapes were tested on the aggregation of an amyloid-β peptide (Aβ(1–40). Recently, Wang et al. (2019) reported that upon incubation of gold nanoparticles with Aβ(1–40), a process of peptide nucleation occurred through interfacial adsorption of Aβ(1–40). It was further explored by spectroscopic tool that with shape-dependent alterations of AuNPs, considerable changes in secondary structure transformation of Aβ(1–40) occurred. In another interesting work, surface-coated, i.e., poly(acrylic acid), NPs revealed potential Aβ aggregation inhibition capacity at a substoichiometric ratio of 1:2,000,000. Such rationally designed aggregation inhibitors hold promise in the future for the treatment of AD. Notable study of Gao et al. (2015) substantiates that efficacy of gold nanoparticles (AuNPs) and nanoclusters (AuNCs) in the inhibition of protein amyloidosis is associated with the size. They showed that large AuNPs have the capacity to accelerate Aβ fibrillation, whereas small AuNPs could suppress the aggregation efficiently. It has been shown that the nucleation and aggregation process of peptides are markedly influenced by nanoparticles (John et al. 2018). Gao et al. (2015) using another chemistry to synthesize inorganic nanoparticles (AuNPs@POMD-pep; AuNPs, gold nanoparticles; POMD, polyoxometalate; pep, peptide) showed its efficacy in the inhibition of amyloid peptide aggregation for the treatment of AD. Kim et al. (2017a, b) developed an in vitro assay to inhibit amyloid-β anti-aggregation using gold nanoparticles. In this approach the gold nanoparticles were used as nucleation cores and optical reporters for efficient detection and inhibition of amyloid aggregation.

12.7 Potentials and Hope of Gold Nanoparticles for Alzheimer's Disease Treatment

The potentials of GNPs for the treatment of AD-associated complications could be strengthened based on the work of Muller et al. (2017). It was shown that GNPs treatment ameliorated STZ-induced impairment of mitochondrial ATP production, neuroinflammation, and oxidative stress in rats. It was even found that STZ-mediated deficits in both spatial and recognition memory were prevented by GNPs. In another facet of work, spherical nanoparticles were shown to inhibit the formation of Aβ fibrils that was achieved using gold nanorods (AuNRs) by influencing the nucleation and growth pathways of fibrillation (Sudhakar et al. 2017). Here, the shape-dependent plasmonic properties of AuNRs and its higher absorbance capacity with near-infrared (NIR) laser light helped the disruption of mature Aβ fibrils. Due to the stabilization of AuNRs by negatively charged lipid (DMPC), inhibition of the amyloid fibrils occurred after selective binding of DMPC-AuNRs to the positively charged amyloidogenic sequence of Aβ protein. In fact, significant amount of Aβ fibrils were broken down into smaller fragments with the impact of NIR and the presence of AuNR particles. Notable work employed lipoprotein-based nanoparticles approach wherein a nanosystem consisting of curcumin fluorescent motif and an NIR-responsive gold core helped aggregation-dependent fluorescence detection and photothermal disassembly of insoluble amyloid aggregates (Martins et al. 2017).

The exceptional physicochemical properties of NPs also help to carry molecules as well as exhibit target functions crucially that could be important in therapeutic point of view. A strategic approach that employed gold nanoparticles tagged with the peptide sequence THRPPMWSPVWP (CLPFFD) has potential in destroying the toxic aggregates of β-amyloid peptide associated with AD (Prades et al. 2012). Actually, this peptide incorporated NPs fused with the transferrin receptor present in the microvascular endothelial cells of the blood-brain barrier, thereby promoting the permeability of the NP conjugates in brain which was revealed by both in vitro and in vivo data.

By applying near-infrared femtosecond (fs) laser in combination with gold nanorods (AuNRs), the preformed amyloid fibrils were disrupted implicating the therapeutic importance of gold nanoparticles in AD treatment (Lin et al. 2016). The effect of AuNPs on behavioral functions (Morris water maze test) in AD rat model provides further information on how the acquisition and retention of spatial learning and memory are improved after NPs therapy (Sanati et al. 2019) Both intrahippocampal (IH) and intraperitoneal (IP) routes of NPs administration improved the cognitive functions by increasing the expression levels of neuronal survival markers such as BDNF (brain-derived neurotrophic factor), CREB (cAMP response element-binding protein), and stromal interaction molecules (STIM1 and STIM2). Through design of hybrid peptide inhibitors (VVIA and LPFFD) and its further attachment onto AuNPs, the interaction of $A\beta_{42}$ peptide was enhanced that led to strong inhibition of Aβ oligomerization and fibrillation process as well as the cytotoxicity of

those species when tested in vitro (Xiong et al. 2017). Using *Caenorhabditis elegans* AD model, Morales-Zavala et al. (2017) explored the therapeutic potentials of nanoconjugates which were described as peptide multifunctionalized gold nanorods with proven capacity to recognize the toxic Aβ aggregates and to efficiently deliver nanorods to the mammalian central nervous system. It showed a better cell penetration capacity without affecting the neuronal viability in vitro and when administered to AD model of *Caenorhabditis elegans*, the toxicity of Aβ peptide was considerably decreased. By employing gold nanoparticle-capped mesoporous silica (MSN-AuNPs)-based H_2O_2-responsive controlled release system, targeted delivery of metal chelator CQ was achieved despite other added advantages such as the high BBB permeability, efficient anti-Aβ aggregation, and good biocompatibility of MSN-CQ-AuNPs for the release of CQ. Overall, the potentials of such NPs to decrease the Aβ self-assembly implicate its importance in AD treatment (Yang et al. 2016). With the assessment of neuroinflammatory and neuroapoptotic markers, Kim et al. (2017a, b) showed that anthocyanin-loaded polyethylene glycol-gold nanoparticles (PEG-AuNPs) had striking neuroprotective effect than the anthocyanins alone treatment in both in vitro and in vivo AD model. In parallel, the same group has revealed that PEG-AuNPs could ameliorate $A\beta_{1-42}$-induced memory impairments, synaptic dysfunctions, tau hyperphosphorylation, apoptosis, and neurodegeneration in AD mice model implicating that combination of dietary polyphenolic compounds with gold nanoparticles could be potential for AD treatment (Ali et al. 2017). Employing the catalytic and optical properties of the AuNPs and the fact that AuNPs could be used for monitoring Aβ aggregates, it has become an important tool in nanotechnology research for testing and screening the anti-Aβ drugs and for pharmacological AD intervention (Lee et al. 2018). The poor solubility of anti-AD drug xanthoceraside was overcome by conjugating xanthoceraside with gold nanoparticles by employing green ultrasonic method wherein silica spheres were used as templates and HF solution as selective etching solvent (Meng et al. 2016). This approach helped improve the solubility of xanthoceraside loaded on hollow gold nanoparticles from 3.0 μg/mL and 2.5 μg/mL to 12.7 μg/mL and 10.7 μg/mL at 25 °C and 37 °C, respectively.

12.8 Conclusion

The gold nanoparticles and its role in the neurodegenerative diseases have been explained in this chapter with suitable research articles. It concludes that the role of gold nanoparticles with its applications is very much important in the future. Due to the eco-friendly nature of the gold nanoparticles, it will play a vital role in the drug delivery across the blood-brain barrier. In the future the gold nanoparticles and its bioconjugated drugs will be widely used in the treatment of neurodegenerative diseases.

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Chapter 13
Nanolipidic Carriers as Potential Drug Delivery Vehicles in Alzheimer's Disease

Sushama Talegaonkar, Namrata Gautam, Venu Varshney, Sandeep Kumar Sharma, and Arundhati Bhattacharyya

Abstract Alzheimer's disease (AD) is the most common cause of dementia accounting for about 60–80% of the cases. With the rise of population of elderly people all over the world, providing greater medical relief to the patients suffering from Alzheimer's disease has become a matter of great urgency. The exact etiology of AD is still unexplained but several hypotheses explaining the pathophysiology of AD have been put forward.

The currently approved pharmacotherapy of AD utilizes cholinesterase inhibitors and NMDA receptor antagonists which provide only symptomatic relief. The drugs used for treatment of Alzheimer's disease should be able to cross the blood-brain barrier (BBB) and reach the central nervous system before the therapeutic effect can be exerted. Therefore, it is a big challenge to design drug delivery system (DDS) capable of targeting drugs to the intended delivery site in the brain.

Lipid-based nanosized drug delivery systems seem to be very promising in delivering the entrapped drug to the brain by virtue of their lipidic nature and small size. Lipid-based nanocarriers have the added advantage of very low cytotoxicity and avoidance of P-glycoprotein-mediated efflux activity of brain endothelial cells apart from other advantages like ability to entrap both hydrophobic and hydrophilic drugs and greater entrapment efficacy. The aim of the present chapter to review the treatment options currently available for Alzheimer's disease and various lipid-based nanocarrier systems explored for enhancing the therapeutic efficacy of anti-Alzheimer drugs along with the challenges in targeting delivery of drugs to the brain.

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13.1 Introduction

With increasing lifespan of people and increasing aging populations around the world, the concerns associated with quality of life also continue to increase. One of the leading causes of compromised quality of life in the geriatric population is Alzheimer's disease (Prince and Jackson 2009). AD is a neurodegenerative disorder characterized by a progressive and irreversible neuronal damage that was described for the first time by the German Physician Alois Alzheimer in the year 1906 (Stelzmann et al. 1995). Accounting for 60–70% of cases of dementia, AD is the leading cause of dementia worldwide (www.who.int). Based on epidemiological data collected in recent years, Alzheimer's Disease International (ADI) estimated incidence of AD in 14th World Health Organization (WHO) regions in 2005. The results showed that North America and Western Europe have the highest prevalence of dementia (6.4 and 5.4% of the population at age 60), followed by Latin America (4.9%) and China and its developing Western Pacific neighbors (4.0%). Compared with Africa, Asia, and Europe, the prevalence of AD was higher in the USA (Ferri et al. 2005).

The nine countries with the largest number of people with dementia in 2010 were China (5.4 million), USA (3.9 million), India (3.7 million), Japan (2.5 million), Germany (1.5 million), Russia (1.2 million), France (1.1 million), Italy (1.1 million), and Brazil (1.0 million).

According to WHO, there were 50 million registered cases of dementia in 2017. Every year, there are nearly 10 million new cases. The total number of people with dementia is expected to reach 82 million in 2030 and 152 million in 2050 globally (www.who.int). Though 1–6% of the AD cases emerge in people aged between 30 and 60 years, which is known as early-onset AD, in 90% cases, AD occurs in people older than 60 years (Mullane and Williams 2013).

Every 65 s someone in USA develops AD (Alzheimer's Association 2018). AD is the 6th leading cause of death in the USA. One in three senior dies with AD in the USA. 16.1 million Americans provide unpaid care to people with AD or other dementias. An estimated 5.7 million Americans of all ages are living with Alzheimer's dementia in 2018. This number includes an estimated 5.5 million people age 65 and older and approximately 200,000 individuals under age 65 who have younger-onset Alzheimer's, though there is uncertainty about the younger-onset estimate (Alzheimer's Association 2006) (Hebert et al. 2013). In the USA, the percentage of people with Alzheimer's dementia increases with age: 3% of people age 65–74, 17% of people age 75–84, and 32% of people age 85 and older have Alzheimer's dementia (Hebert et al. 2013).

Dementia which is a characteristic symptom of AD causes disability and dependency in patients all over the world. It is caused by an abnormal aging of the central nervous system (CNS) with decrease in cognitive function, memory, thinking ability, reasoning, and learning (Ferretti et al. 2018). Dementia severely impacts the work and social life of the persons as the affected persons find it difficult to express themselves. With the progression of the disease, the patients require extensive help with their daily activities as well (Alzheimer's Association 2012).

According to the WHO report 2008, treating and caring for people with dementia costs the world more than US$ 604 billion per year (World Health Organization 2008). This includes the cost of providing health and social care as well as the reduction or loss of income of people with dementia and their caregivers. In Europe it is estimated that the future cost of dementia would rise by approximately 43% from 2008 reaching 250 billion Euros in 2030.

The aim of the present chapter is to provide a brief overview of AD with underlying pathophysiology and the currently available treatment modalities. The chapter also reviews in detail the potential role that lipid-based nanocarriers can play in increasing the efficacy of the anti-Alzheimer drugs by selectively delivering these to the central nervous system (CNS).

13.1.1 Stages of Alzheimer's Disease

The stages are separated into three categories: mild Alzheimer's disease (early stage), moderate Alzheimer's disease (middle stage), and severe Alzheimer's disease (late stage). The pace at which symptoms advance from mild to moderate to severe varies from person to person (Fig. 13.1). On average, a person with Alzheimer's has lifespan of 4–8 years after diagnosis, but he can live as long as

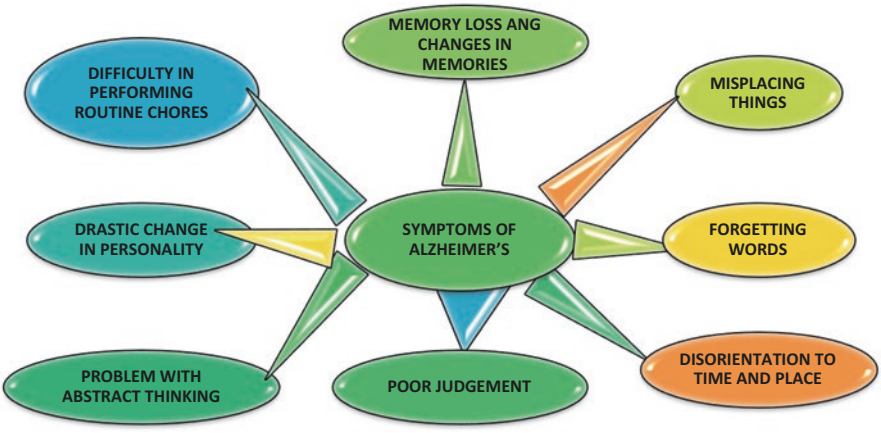

Fig. 13.1 Symptoms of Alzheimer's disease

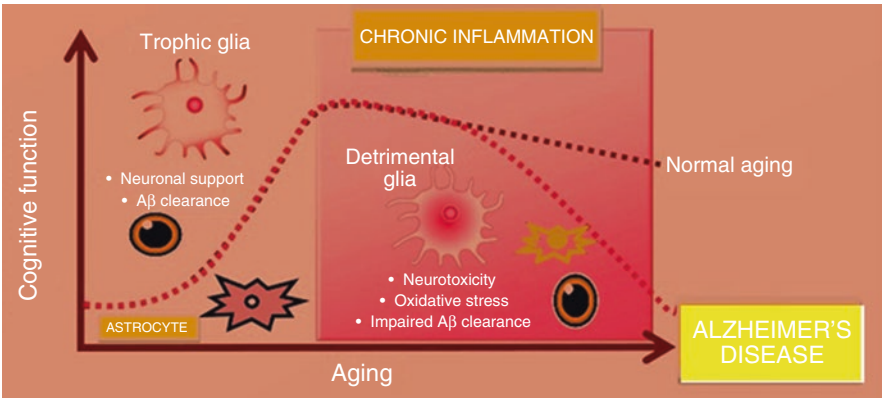

Fig. 13.2 Cognitive function with progression of Alzheimer's disease

20 years as well. Despite the categorization, practically it is difficult to put a person with Alzheimer's in a specific stage as stages may overlap. Figure 13.1 illustrates the different symptoms of AD and the decline in cognitive function in persons with AD vis-à-vis normal aging is illustrated in Fig. 13.2.

Mild Alzheimer's Disease (Early Stage)
The early stage is often mistaken. Relatives and friends (and sometimes professionals as well) confuse it with "old age." Because the onset of the disease is gradual, it is difficult to be sure exactly when it begins. Although the early stage patients can function independently, they may have difficulties like problems in having communication, i.e., difficulty in coming up with the right word or name; forgetfulness in remembrance of names when introduced to new people; impaired retention of content once read, heard, visualized, or written; lost abilities of planning or organization; and loss of track of the time, including day, month, year, etc.

Moderate Alzheimer's Disease (Middle Stage)
Moderate Alzheimer's is typically the longest stage and can last for many years. In this stage, the dementia symptoms are more noticeable to others and include: forgetfulness of events and one's own personal history, erratic mood and behavior, no interest in activities and hobbies, severe mood swings including depression or anxiety, and unable to recall their own address or telephone number and high school or college from which they graduated. Some patients feel trouble in controlling bladder and bowels also. Behavioral change like wandering, repeated questioning, calling out, clinging, disturbed sleeping, and hallucinations (seeing or hearing things which are not there) increases. Sleep pattern (circadian cycle) is lost and there are also chances of wandering and becoming lost. The patients also may require help at this stage for their personal care (i.e., toileting, washing, and dressing).

Severe Alzheimer's Disease (Late Stage)
In the final stage of this disease, dementia symptoms are severe. Individuals lose the ability to carry on a conversation and, eventually, to control movement. They may

still say words or phrases, but communicating becomes difficult. As memory and cognitive skills continue to worsen, significant personality changes may take place and individuals need extensive help with daily activities. At this stage, individuals may:

- need round-the-clock assistance with daily activities and personal care such as bathing and toileting
- lose awareness of recent experiences as well as of their surroundings
- experience changes in physical abilities, including the ability to walk (may be unable to walk or be confined to a wheelchair or bed), sit, and, eventually, swallow
- become vulnerable to infections, especially pneumonia
- be usually unaware of time and place
- have difficulty understanding what is happening around them
- be unable to recognize relatives, friends, and familiar objects
- have bladder and bowel incontinence
- have behavioral changes which may escalate and include aggression and nonverbal agitation (kicking, hitting, screaming, or moaning)
- be unable to find his or her way around the home

13.1.2 Risk Factors for Alzheimer's Disease

Experts believe that just like other diseases Alzheimer's develops as a result of multiple factors rather than a single cause. Some risk factors are discussed here.

Age—The percentage of people with Alzheimer's dementia increases dramatically with age: 3% of people aged 65–74, 17% of people aged 75–84, and 32% of people age 85 or older have Alzheimer's dementia (Hebert et al. 2013) but that does not imply that if a person is older he will develop Alzheimer's dementia (Fig. 13.2).

Family History—Individuals having parents or siblings with Alzheimer's are more likely to develop AD than those who do not have a first-degree relative with AD (Green et al. 2002).

APOE-e4 Gene—Everyone inherits one of three forms of the APOE gene: ε2, ε3, and ε4 from each parent. Having the ε4 form increases the risk of developing Alzheimer's Disease (Mahley and Rall 2000) and is more likely to develop Alzheimer's at a younger age (Spinney 2014).

Familial/Early Onset—Familial Alzheimer's Disease which develops before age 60 is due to mutations in the amyloid precursor protein (APP) and/or presenilin 1 and 2 gene (PSEN1 and PSEN 2).

Cerebrovascular Disease—According to Pendlebury and Rothwell (2009), there is twofold increased risk of dementia after first incident stroke. The mechanisms were believed to be destruction of brain parenchyma with atrophy, an increase in Aβ deposition, and the combination of vascular and Alzheimer-type pathology (Blennow et al. 2006).

Hypertension—Not only high but abnormally low blood pressure is also associated with dementia (Waldstein et al. 2005). In clinical trials, AD patients were given antihypertensive medications but the results were inconsistent (Lithell et al. 2003).

Type II Diabetes—Type II diabetes increases the risk of AD by twofold (Luchsinger et al. 2004). Reger et al. (2008) showed that the administration of intranasal insulin improved cognition in the patients who were in the early phases of AD. Same results were reported in a 6-month trial of the PPAR-g agonist, rosiglitazone, by Watson et al. (2005).

Smoking—Smoking either increases the risk of AD or there is no association (Doll et al. 2000). Nicotine increases acetylcholine release, elevates the number of nicotinic receptors, and improves attention but it also increases oxidative stress which contributes to AD (Rottkamp et al. 2000; Koponen et al. 2004).

Traumatic Brain Injury—Individuals having suffered traumatic brain injury have a higher risk of dementia, particularly those who carry the APOE-e4 allele (Koponen et al. 2004). Postmortem and experimental studies show that after human brain injury, both Aβ deposition (Hartman et al. 2002) and intraneuronal tau pathology are increased, even in younger patients (Smith et al. 2003). Higher levels of education, physical activity, and Mediterranean diet on the other hand were shown to decrease the risk of developing AD.

13.1.3 Diagnosis of Alzheimer's Disease

The revised guidelines of the National Institute on Aging (NIA) and the Alzheimer's Association (2012) incorporate some biomarker tests. A biomarker is a measurable indicator for the presence or absence of a disease or the risk of developing a disease. For example, blood glucose level is a biomarker of diabetes, and high blood pressure is a biomarker of heart disease risk. Some biomarkers for Alzheimer's are the amount of beta-amyloid in the brain as shown on positron emission tomography (PET) imaging, levels of certain proteins in fluid (e.g., levels of β-amyloid and tau in the cerebrospinal fluid and levels of particular groups of proteins in blood), and level of glucose metabolism in the brain as shown on PET imaging using the radiotracer fluorodeoxyglucose (Hyman et al. 2012).

13.1.4 Preclinical Alzheimer's Disease

In 1984 it was thought that AD begins when symptoms of dementia such as memory loss are already present and individuals fail to carry out daily tasks but due to revised guidelines it came to light that preclinical Alzheimer's disease is silent stage of AD in which individuals have no symptoms of memory loss but have measurable changes in the brain, cerebrospinal fluid, and/or blood (biomarkers) that indicate the earliest signs of disease showing that brain changes in

AD may begin 20 years or more before symptoms occur (Reiman et al. 2012; Bateman et al. 2012; Villemagne et al. 2013).

13.1.5 Mild Cognitive Impairment (MCI): A Potential Precursor to Alzheimer's and Other Dementias

MCI affects 15–20% of people age 65. In this condition, mild but measurable changes in thinking abilities can be easily noticed by the family members and friends of the person affected (Roberts and Knopman 2013). People with MCI are more likely to develop Alzheimer's than people without MCI (Kantarci et al. 2009). Revised guidelines suggest that if a person has MCI symptoms along with elevated levels of beta-amyloid, the individual may be in an early stage of Alzheimer's (called MCI due to Alzheimer's disease) (Sperling et al. 2011; Albert et al. 2011). However, MCI can develop for reasons other than Alzheimer's, and MCI does not always lead to dementia. In some individuals, MCI reverts to normal cognition or remains stable.

13.2 Etiology of Alzheimer's Disease

Though the cause of AD is still not fully understood, the complexity of AD pathophysiology has led researchers to propose several hypotheses that might contribute to the genesis of this disease. The most popular among these are the amyloid hypothesis and the tau hypothesis.

13.2.1 The Amyloid Hypothesis

The amyloid beta (Aβ) hypothesis is most widely accepted to explain the pathophysiology of AD. It states that Aβ deposition is the main and important causative factor of AD. Aβ is generated by the proteolysis of amyloid precursor protein (APP) which is a type I single-pass transmembrane protein expressed at high levels in the central nervous system (CNS). Though the exact physiological function of APP is not known, it is suggested to have a role in signaling pathways in the brain which includes synapse formation, neurogenesis, axonal transport, cell signaling, and plasticity (Thinakaran and Koo 2008). APP undergoes sequential proteolysis. It is first cleaved by α-secretase (nonamyloidogenic pathway) or β-secretase (BACE1) (amyloidogenic pathway) and then by γ-secretase (Vassar 2004). Non-amyloidogenic pathway is basically nontoxic and it starts by the cleavage of APP by α-secretase which generates a soluble sAPPα and a membrane-anchored C-terminal fragment C83. The C-terminal fragment (C83) is further cleaved by a γ-secretase to produce

a short fragment P3 and an APP intracellular domain (AICD). For the amyloidogenic pathway, APP is cleaved by β-secretase to produce sAPPβ and a C-terminal fragment containing 99 amino acids (C99). C99 is further cleaved by γ-secretase to form Aβ40 or Aβ42 fragment (Sahni 2011). Aβ peptide also has a tendency to form oligomers. Oligomers can form Aβ fibrils and protofibrils that will eventually form amyloid plaques preferably in the cerebral cortex and the hippocampus area of the brain (Golde 2005). These plaques activate microglial cells and astrocytes which trigger the release of inflammatory cytokines and chemokines leading to neuroinflammation and neuronal damage (Dzamba et al. 2016). Amyloid oligomers cause neurotoxicity and initiate the amyloid cascade. The elements of the cascade include local inflammation, oxidation, excitotoxicity (excessive glutamate), and tau hyperphosphorylation which ultimately results in cell death (Golde 2005). Healthy individuals do not have amyloid-induced cytotoxicity because of the fact that they can clear amyloid from the brain before it reaches neurotoxic levels by balancing amyloid production with its clearance (Hardy and Selkoe 2002).

It also needs to be mentioned that there are certain mutations in the presenilin 1 (PS1) and presenilin 2 (PS2) genes which account for most of the cases of familial early-onset AD (FAD) occurring in 30–50-year-old patients. The mutations in PS1 and PS2 increase the activity of γ-secretases. Because of increased γ-secretase activity, proteolysis of APP is also increased leading to enhanced Aβ formation, which is a characteristic of AD (Gopal 1999).

13.2.2 Tau Protein

Phosphorylated tau proteins are not causative factor for the disease but the reflection of neuro-damage (Hardy and Selkoe 2002). The inflammation produced by Aβ accumulation would lead to hyperphosphorylation of the microtubule-associated protein tau (De Paula et al. 2009). Tau protein is a microtubule-associated protein (MAP), which binds to the microtubules and stabilizes them (Weingarten et al. 1975). In the brain of AD patients, hyperphosphorylation of tau protein is at least three times higher than that in the normal brain (De Paula et al. 2009). This abnormal hyperphosphorylation causes the tau protein molecules to move away from the microtubules and misfold to stick to each other, ultimately forming paired helical filament (PHF) tau and neurofibrillary tangles (NFTs) (Gong and Iqbal 2008). NFTs negatively affect neurotransmitter transport and axonal integrity. This may ultimately lead to neurodegeneration in AD patients.

13.2.3 Cholinergic-Deficit Hypothesis

Rossor et al. (1980) and Henke and Lang (1983) reported that the brains of AD patients showed not only degeneration of cholinergic neurons but also reduction in cholinergic markers such as choline acetyltransferase (ChAT), the enzyme respon-

sible for the synthesis of acetylcholine (ACh). This led to the so-called cholinergic-deficit hypothesis of AD. Moreover, phospholipase A2 (PA2) enzyme which is responsible for the conversion of phosphatidylcholine to choline (Gattaz et al. 2014) has been reported to decrease in the frontal and parietal cortexes of AD patients and because choline is converted to acetylcholine by ChAT and AChE, its deficiency leads to cholinergic deficiency and AD progression. A study by Soininen et al. (1995) also showed that those AD patients which have the apolipoprotein E (APOE) ε4 allele have a more severe cholinergic deficit than the AD patients without the APOE ε4 allele.

13.2.4 Glial Cell Involvement in AD Pathophysiology

Glial cell-mediated inflammation plays an important role in AD pathophysiology. Four groups of glial cells are believed to be involved in AD pathophysiology: microglial cells, oligodendrocytes, NG2 glial cells, and astrocytes (Morales et al. 2014). Amyloid plaques or senile plaques activate microglial cells in their proximity which when activated performs the phagocytosis of cell debris or foreign particles and cytokines to protect CNS (Fu et al. 2014). Oligodendrocytes or oligodendroglia are a type of neuroglia whose main functions are to provide support and insulation to axons in the central nervous system by providing myelin sheaths which allow the fast propagation of action potentials, but when oligodendrocytes are in the vicinity of the amyloid or senile plaques, they release iron contained inside them. Iron is directly involved in myelin production and its deficiency in oligodendrocytes causes myelin breakdown. This promotes Aβ oligomerization and deposition, potentiating Aβ toxicity (Bartzokis et al. 2007). There exists another group of glial cells termed oligodendroglial precursor cells (OPCs) in the brain which express NG2 (a chondroitin sulfate proteoglycan) and are therefore called NG2 cells (Xu et al. 2011). NG2 cells are majorly responsible for Aβ uptake and its clearance by the lysosomal pathway (Li et al. 2013). In patients suffering from Alzheimer's, the number of NG2 cells is reduced which diminishes Aβ clearance and thus increases its deposition contributing to AD progression. Astrocytes are widely distributed throughout the CNS, playing roles such as elimination of neuronal debris, excitability of neurons, defense against oxidative stress through production of glutathione, prevention of neuronal toxicity by glutamate homeostasis, and synaptic development and plasticity (He and Shen 2009; Finsterwald et al. 2015; Khakh and Sofroniew 2015). In AD, there is marked oligomeric amyloid-β generation which gives rise to astrocytes with a reactive phenotype and thus there is abnormal regulation of the processes mediated by astrocytes. This results in multiple negative outcomes which include glutamate excitotoxicity, impaired synaptic plasticity, oxidative stress, etc. (Crystal et al. 2017).

13.2.5 Oxidative Stress in Alzheimer's Disease

Oxidative stress occurs because of variety of molecules and free radicals derived from molecular oxygen collectively called reactive oxygen species (ROS). Under normal conditions, there is a balance between ROS formation and antioxidant. In various pathological scenarios including the AD, antioxidant defense system of the cells is not able to cope with oxidant species which generates oxidative stress. Thus, ROS starts oxidizing many cell structures and molecules which deteriorates them and leads to aging. Other important reasons for increased ROS production are mitochondrial dysfunction and chronic inflammatory responses occurring in AD. Apart from this, oxidative stress also increases β-secretase and γ-secretase activity, thus increasing Aβ formation (Cervellati et al. 2016).

13.2.6 Apolipoprotein E and Alzheimer's Disease

Apolipoprotein E (ApoE) produced by astrocytes in CNS is majorly involved in lipid transport and injury repair in the brain. The ε4 allele of the APOE is the strongest risk factor for late-onset AD and ε2 form may decrease one's risk (Corder et al. 1993). All ApoE isoforms have different ability to bind lipids and Aβ. The ε3 form is the most common. The ε4 form is the next most common, and the ε2 form is the least common (Mahley and Rall 2000). It has also been shown in some studies that APOE genotypes vigorously show deposition of Aβ to form senile plaques and cause cerebral amyloid angiopathy (CAA) (Ellis et al. 1996). The Aβ deposition in the form of senile plaques is more abundant in APOE ε4 carriers compared with noncarriers (Kok et al. 2009).

13.2.7 NMDA (Glutamate) Receptor

The N-methyl-D-aspartate receptors (NMDARs) are cationic channels gated by the neurotransmitter glutamate which play an essential role in excitatory transmission, learning, and memory in the central nervous system (CNS) (Kamat et al. 2013). Glutamate levels are maintained in the CNS by astrocytes which uptakes and metabolizes excessive glutamate from synaptic cleft. In AD patients, the ability of astrocytes to uptake and metabolize glutamate is decreased, causing chronic excess of glutamate levels and thus overactivation of NMDA receptors. This causes excessive calcium (Ca2+) influx in response which causes mitochondrial functional impairments and ROS formation (Koleske 2013). ROS oxidizes many cell structures and molecules leading to aging while sudden and excessive Ca2+ influx causes series of events leading to cell death including neurotoxicity. Thus, improper NMDA receptor may participate in the pathogenesis of AD (Kamat et al. 2013).

13.3 Treatment for Alzheimer's Disease

The pharmacotherapy of AD utilizes cholinesterase inhibitors and NMDA receptor antagonists which provide only symptomatic relief. Permanent cure of AD still remains elusive. It is imperative that drugs used for treatment of Alzheimer's disease should be able to cross the blood-brain barrier (BBB) and reach the central nervous system before the therapeutic effect can be exerted. The first choice for the treatment is choline esterase inhibitors (ChEIs) which are meant to prevent degradation of acetylcholine in the synaptic cleft, important for learning and memory. Widely used ChEIs include oral tacrine, donepezil, rivastigmine, galantamine, as well as rivastigmine patches. It has also been hypothesized that in AD, glutamate causes excessive and nonphysiological activation of NMDA receptors, thus causing excitotoxic neuronal damage. In 2003, the USFDA approved memantine, a moderate and noncompetitive NMDA receptor antagonist for the treatment of moderate to severe stages of AD. A combination of one of the cholinesterase inhibitors, e.g., donepezil with memantine, is also being prescribed.

13.3.1 Disease-Modifying Agents

Sixty-three percent of drugs which are under testing or trials are called disease-modifying therapies (DMTs). This means that they not only work to reduce current symptoms, but rather to improve outcome over a longer period of time. Most of the DMTs which are under trials work either to reduce amyloid levels in the brain or to decrease its production or they work on tau proteins (Cummings 2017). The major categories of disease-modifying agents are described in the following section.

13.3.1.1 Amyloid Treatment

Various approaches have developed to slow or prevent amyloid aggregation and improve clearance from the brain. These include immunotherapy and enzyme inhibitors. Some of the anti-amyloid approaches are described below.

Active and Passive Immunotherapy
In transgenic AD mouse models, anti-Aβ antibodies were generated by active immunization. It was seen that at the preclinical AD stage or at the onset of AD-like pathogenesis, brain Aβ levels were lowered significantly but with well-established AD-like pathology, effects were variable. A second-generation safer vaccine, ACC-001, is currently in phase II clinical trials in patients with mild to moderate AD and it has no adverse effects such as aseptic meningoencephalitis which were observed in 6% of patients after the administration of first-generation amyloid vaccine AN-1792 (Lemere and Masliah 2010).

Monoclonal Antibodies (mAbs)
mAb*s* are antibody solutions which are injected intravenously. These are highly specific to the Aβ deposits in the brain and therefore initiate an immune response against them by increasing their uptake by the microglial cells (Robert and Wark 2012). For this to happen, anti-Aβ antibodies should cross the BBB and bind Aβ within the CNS or by "sink effect," it could bind with Aβ peptide in the blood that would "draw" the peptide from the brain to the periphery through the BBB (DeMattos et al. 2001). Bapineuzumab, crenezumab, gantenerumab, solanezumab, and others are the drugs belonging to this class (Robinson 2015). A phase II clinical trial of bapineuzumab showed that it decreased both total and phosphorylated tau levels in CSF but did not affect Aβ level with adverse effects being transient cerebral vasogenic edema in some patients. Most of immunotherapy decreased cognitive decline and reduced beta-amyloid load, but the adverse events still need to be solved.

BACE Inhibitors
Drugs have been developed to reduce beta-amyloid production that inhibit the activity of an enzyme called β-site APP-cleaving enzyme (BACE) which generates beta-amyloid protein from APP. Among the BACE inhibitors in testing are verubecestat, LY3314814, CNP520, and others. Small molecule inhibitors of secretases are nonspecific, while larger molecules which are more specific have very less BBB permeation (Cummings 2017). These agents are only useful if started early in the disease process, which is well before most AD patients are diagnosed.

γ-Secretase Inhibitors/Modulators
γ-Secretase inhibitors like DAPT decreased Aβ levels in plasma and cerebrospinal fluid (CSF) of AD mice/rats. Another γ-secretase inhibitor semagacestat (LY450139) dihydrate reduced Aβ levels in serum but not in the CSF. Notch, which is necessary for growth and development, is also a substrate of γ-secretase. Notch-related side effects of γ-secretase inhibition (e.g., severe gastrointestinal and hemopoietic side effects, neurodegeneration) are the biggest problems in developing useful γ-secretase inhibitors. Thus, there is a shift of drug development toward γ-secretase modulators (Karran et al. 2011).

α-Secretase Activators/Modulators
Since α-secretase and β-secretase work on the same substrate APP, Aβ secretion can be decreased by upregulating the activity of α-secretase which will decrease the amount of APP available for β-secretase and thus have therapeutic potential. Members of the adamalysin family of proteins, mainly ADAM 10, ADAM 17, and ADAM 9, fulfill some of the criteria required of α-secretase. ADAM10 was overexpressed in transgenic mice which showed less amyloid deposition as well as improved neurological function (Postina et al. 2004). Deprenyl and PKC activator TPPB can also increase α-secretase activity and decrease Aβ secretion (Yang et al. 2009). This implies that stimulating α-secretase may have benefit but no clinical data is available at present.

Peptide Inhibitors of Amyloid Aggregation
Tjernberg et al. (1996) used amyloid peptide fragment KLVFF as an aggregation inhibitor. Although aggregation was still seen, a significant decrease in fibrillization led to designing of another peptide inhibitor, called OR2, which was designed from the KLVFF sequence and could modify early aggregation of Aβ as well as protect SHSY-5Y cells from Aβ cytotoxicity (Pallitto et al. 1999).

M1 Muscarinic Agonists
Activation of M1 mAChRs with agonists leads to either enhanced secretion of sAPPα (via α-secretase activation) or decreased Aβ (via γ-secretase inhibition). Talsaclidine is M1 agonist that stimulates α-secretase activity in vitro so when given to AD patients in a clinical study, it decreased CSF Aβ about 20% compared with the baseline (Hong 2012).

Aβ Aggregation Inhibitors
Intra-hippocampal injection of β-sheet breaker iAβ5p not only improved memory but also decreased amyloid plaques (Hong 2012). Tramiprosate also inhibited the formation of neurotoxic aggregates in the brain but it failed in US phase III trial in 2007. Resveratrol, myricetin, morin, tannic acid, curcumin, ferulic acid, nordihydroguaiaretic acid (NDGA), and (−)-epigallocatechin gallate (EGCG) had strong anti-Aβ aggregation effects in vitro. Colostrinin (CLN) isolated first from ovine colostrums improved learning, memory, and cognitive functioning as it inhibited the aggregation of Ab peptides and dissolved pre-formed fibrils.

Aβ-Degrading Enzymes
Studies show that Aβ peptide can be degraded by proteases called Aβ-degrading enzymes like neprilysin (NEP), insulin-degrading enzyme (IDE), plasmin, endothelin-converting enzyme (ECE) 1 and 2, and angiotensin-converting enzyme (ACE). Less Aβ degradation and declining cognition was seen in NEP inhibitor injected and/or NEP knockout mice, while overexpression improved spatial memory and decreased Aβ levels. Studies have shown that APP intracellular domain (AICD) could upregulate NEP transcription and thus increase Aβ degradation (Belyaev et al. 2009). Imatinib was shown to elevate AICD in H4 human neuroglioma cells and thus increase of NEP activity as well (Bauer et al. 2011).

13.3.1.2 Treatments Based on Tau Pathology

Tau phosphorylation increases drastically in AD, indicating tau kinase inhibitors could be used as an anti-AD treatment. Tau aggregation inhibitors and immunotherapy also could be viable approaches for AD therapy.

Glycogen Synthase Kinase (GSK)-3β
It is well established that this kinase can phosphorylate tau in cells in culture and in the brains of transgenic mice. In animal models kinase is blocked by lithium, preventing tau phosphorylation (Roberson and Mucke 2006). The M1 muscarinic agonist AF267B also inhibits GSK-3β activity and thus reduces tau phosphorylation in

transgenic mice. Two additional inhibitors are propentofylline (PPF) and SRN-003-556. Finally, activated MAPK has been reported to be associated with neurofibrillary tangles (NFTs) in human AD (Husain et al. 2008). Its inhibitors could have a role in AD treatment.

Preventing Tau Aggregation
Studies show that some inhibitors not only prevent tau protein aggregation but can also dissolve the already formed aggregates, which include phenothiazines, anthraquinones, polyphenols, thiacarbocyanine dyes, thiazolyl-hydrazides, rhodanines, aminothienopyridazines, and so on (Ballatorea et al. 2011). Studies in vivo are still needed to find the efficacy and safety of tau aggregate inhibitors.

Prevention of the Misfolding of Tau
Misfolding of hyperphosphorylated tau proteins also contributes to AD. It is well known that heat shock protein 90 (Hsp 90), a chaperone, folds the denatured proteins and it has a role in preventing tau degradation under normal conditions. Curcumin is also reported to inhibit Hsp 90 which under pathological conditions degrades tau in spite of preventing it (Giommarelli et al. 2010).

Tau Immunotherapy
Asuni et al. (2007) demonstrated that immunization of mice expressing P301L-tau (JNPL3 mice) with a small phospho-tau peptide resulted in the production of antibodies that entered the brain and slowed the progression of the behavioral phenotype. Thus a passive immunization may be a better therapeutic approach.

13.3.2 Oxidative Stress and Antioxidants

Acrolein, the by-product of lipid peroxidation, as well as markers of oxidative stress such as heme oxygenase-1 was found to be elevated in brains from patients with AD, which indicates that oxidative damage has an early role in the pathogenesis of AD (Schipper et al. 2006; Nam et al. 2010). Interestingly, in vitro and in vivo studies reported that oxidative markers are decreased by the administration of different polyphenolic compounds such as catechins, curcumin, or resveratrol (Singh et al. 2008).

Catechins
Green tea which belongs to a class of polyphenol has epigallocatechin gallate (EGCG) as the main active component besides (−)-epigallocatechin (EGC), (−)-epicatechin (EC), and (−)-epicatechin-3-gallate (ECG) (Moyers and Kumar 2004). Green tea extract protects neurons from the Aβ-induced damages because ECGC modulates various pathways such as MAPK, PKC, and phosphatidylinositol-3-kinase (PI-3 kinase)-Akt (Chen et al. 2001; Levites et al. 2003; Koh et al. 2003). Though its instability in solution and degradation through oxidative processes needs to be resolved.

Curcumin

It is used as spice in India and it is extremely safe even at very high doses. Curcumin blocked Aβ aggregation in vitro (IC50 = 0.8 μM). In vivo, in Tg2576 mice, reduction of amyloid plaque burden was observed after curcumin treatment (Yang et al. 2005). Moreover, curcumin could chelate the redox active metal iron and copper which have a role in AD pathogenesis. However, its extremely low aqueous solubility, rapid systemic elimination, and inadequate tissue absorption, which severely retards its bioavailability need to be addressed (Anand et al. 2007).

Resveratrol

Resveratrol (trans-3,4,5-trihydroxystilbene) is the main biologically active non-flavonoid found in grapes and red wine (Baum and Ng 2004). Some studies show less incidence of AD with increasing wine consumption. Not only resveratrol protected PC12 cells against Aβ-induced toxicity but the secretion of Aβ was also reduced in two cell lines, HEK 293 and N2a (Jang and Surh 2003; Marambaud et al. 2005). Its protective effect may be due to specific activation of Sirt1 (Kaeberlein et al. 2005). However, it is rapidly metabolized in liver and intestinal epithelial cells so its bioavailability needs to be addressed.

In summary, polyphenolic compounds such as catechins, curcumin, or resveratrol are safe and have protective properties but their efficacy in humans is not yet definitively proved as no clinical trials have been completed yet.

13.3.3 Chelating Agents

Concentration of metal ions such as copper (390 μM), zinc (1055 μM), and iron (940 μM) are elevated by several-folds in AD brain as compared to normal samples [copper (70 μM), zinc (350 μM), and iron (340 μM)] (Adlard and Bush 2006). Zinc and the iron regulatory protein-2 have been found to co-localize with NFT-containing neurons. Ferric ions and cupric ions bind to various "repeat" motifs on tau increasing its phosphorylation and aggregation. Therefore, metallo-complexes are emerging as a new target for AD. It has been observed in phase 2 clinical trial that clioquinol reduces the rate of cognitive loss due to its ability to chelate zinc and copper associated with amyloid plaques (Hong 2012).

13.3.4 Nicotine

Nicotine is a cholinergic agonist to release acetylcholine, which is an alkaloid derived from the leaves of tobacco plants (Graham et al. 2002). Nicotinic receptor densities are decreased in neurodegenerative disorders such as AD. Nicotine showed significant improvements in several cognitive tasks and in mood although not on memory when injected on people with AD (Court et al. 2005). It is also believed that

nicotine has a preventive action on AD. Adverse effects of cardiovascular risks in elderly people, sleep, and behavior need to be worked to further study the use of nicotine in patients with AD.

13.3.5 Melatonin (N-Acetyl-5-methoxytryptamine)

It is a neuroprotective tryptophan metabolite, synthesized by the pineal gland. It regulates circadian rhythms, removes free radicals, etc. (Wu and Swaab 2005). In AD patients, there are decreased levels of melatonin in serum and in CSF (Rosales et al. 2012). It inhibits the amyloid beta aggregation as well as prevent the hyperphosphorylation of the tau protein in rats indicating that melatonin may be used in AD (Wang et al. 2005).

13.3.6 Cell Transplantation and Gene Therapy

As cholinergic hypothesis is associated with the pathology of AD, transplantation of cholinergic-rich tissue or peripheral cholinergic neurons was done in AD rat model which improved behavior and cognitive function (Chen et al. 1997). Lack of endogenous nerve growth factor (NGF) can lead to memory deficits so fibroblasts genetically modified to express human NGF were transplanted into the forebrain of eight patients with mild AD and it was seen that cognitive decline was improved as evidenced by the MMSE and AD Assessment Scale. Cerebrolysin 1 (Ever Neuro Pharma) which possesses neurotropic properties has been combined with AChEI and it was shown to have synergistic effects in AD (Allegri and Guekht 2012).

13.3.7 Cholinergic Precursors

Cytidine 5′-diphosphocholine (CDP-choline) and choline alphoscerate are precursors of choline and increase acetylcholine content and release. CDP-choline 9 (citicoline) is a prescribed drug in several European countries and in Japan. Further studies are required on CDP-choline efficacy on memory. Choline alphoscerate can probably cross the BBB and enter nerve cell membranes within 24 h of absorption. A review of 13 clinical trials concluded that it should be confirmed in future investigations for dementia (Sahni 2011).

13.3.8 Monoamine Oxidase (MAO) Inhibitors

MAO inhibitor deprenyl besides being an anti-Parkinson drug has also been used in AD for many years. It is known through in vitro experiments that deprenyl has a role in APP processing through PKC and mitogen-activated protein kinase (MAPK) signaling pathways. Another MAO-B inhibitor rasagiline also inhibits acetylcholinesterase besides APP processing, through PKC and MAPK pathways. Ladostigil is a dual acetylcholine butyrylcholinesterase. It increases cholinergic neurotransmission and is also a brain selective MAO-A and MAO-B inhibitor. It thus shows neuroprotective effects in vivo in scopolamine-induced impairment in spatial memory (Weinreb et al. 2012).

13.3.9 Miscellaneous Agents

Medications for noncognitive behavioral symptoms such as apathy, agitation, and sleep disturbances include the antidepressants escitalopram and mirtazapine, the cannabinoids nabilone and dronabinol, the anticonvulsants carbamazepine and levetiracetam, the novel antipsychotic pimavanserin, the combination of dextromethorphan and quinidine, the mood regulator lithium, and the stimulant methylphenidate. The use of other agents like nonsteroidal anti-inflammatory drugs (NSAIDs) such as celecoxib or indomethacin, phenserine, statins, tarenflurbil, tramiprosate, *Ginkgo biloba*, vitamin E, selegiline, estrogens, and pentoxifylline for the treatment of AD is not recommended yet because of inconsistent results. However, there have been several trials but more studies are still needed to establish whether or not they have any role in treatment of AD (Grossberg 2019). For instance, estrogen when given during hormone replacement therapy (HRT) has also worked as a neuroprotective agent for AD. However, Shumaker and colleagues reported in a study that postmenopausal women treated with estrogen plus progestin were at increased risk for dementia. Thus, for further investigations, studies in AD with estrogen analogues (e.g., premarin, raloxifene) are now mostly in phase II (Shumaker et al. 2003).

The dye methylene blue prevents tau interactions as well as inhibits Aβ aggregation, decreases oxidative stress, prevents mitochondrial damage, and inhibits AChEs. As Aβ oligomers activate various intracellular pathways, drugs that interrupt these signaling pathways could be useful in AD. Recently, it has been reported that rolipram which is a PDE-4 selective inhibitor effectively reversed memory defects in Ab-treated mice. Some studies have reported that if chronic growth factor is deprived for a longer duration, the GABA transmission changes from inhibitory to excitatory stimulus. SGS742 is a GABAB antagonist which is in phase II trial stage and showed good results in phase I trials. Areas of the brain involved in learning and memory have high levels of 5-HT1A, 5-HT4, 5-HT6, and 5-HT7 receptors.

Many 5-HT4 agonistic compounds like PRX-03140, velusetrag, and others have positively affected cognition in animal models as well as affected amyloid processing. Neural stem cell (NSC) engraftment is believed to have a role in AD since NSCsβ are responsible for producing various factors such as neurotrophic factors that promote the regeneration of the CNS (Blurton-Jones et al. 2009; Kumar 2015). Administration of DHA in mild to moderate AD showed no delay in the rate of cognitive decline according to the MMSE.

Despite existence of knowledge regarding this complex disease, very few options are available for its management. BACE inhibitors offer a promising approach, but only few drugs have undergone clinical trials as of now. γ-Secretase, another enzyme involved in Aβ production, can also be targeted but side effects associated with notch inhibition are posing problems. So far, the vaccination approach remains promising because of behavioral improvements in mice. Some novel approaches such as DNA vaccination, NOS modulation, or caspase inhibition are also available but they still are needed to be studied. Peptide-based inhibitors present an alternative and appealing preventative strategy to MAb therapies, as they are not as costly to produce, versatile, and intrinsically safer and can be easily modified for superior BBB permeation.

13.4 Drug Delivery Approaches for Targeted Delivery of Anti-Alzheimer Drugs

Although a variety of therapeutic approaches have been tried in treatment of AD, very few have been successful in the clinical trials. The main reasons attributable to the therapeutic failure of many such drug candidates are poor or low oral bioavailability and inability to cross the blood-brain barrier (BBB). Conventional delivery of acetylcholinesterase inhibitors (AChEIs) like tacrine, donepezil, and rivastigmine is associated with side effects attributable to peripheral cholinergic effects. Most frequently reported side effects include nausea, vomiting, and diarrhea which many a time lead to discontinuation of therapy (McGleenon et al. 1999). Tacrine has been discontinued in the US market since 2013 owing to safety concerns. Brain-targeted delivery can be a viable option for delivery of the therapeutic moiety directly to its target site without any peripheral side effects. In the recent times nanotechnology has emerged as a potential tool for targeted delivery of drug molecules to its site of action including the central nervous system. The potential advantages that nanoparticulate carriers can offer include long circulation half-life, controlled release of the encapsulated drug, ability to cross biological membranes intact, and amenability to surface modification and ligand attachment for targeting. Emergence of nanotherapeutics has contributed a lot in development of various nanosystems like liposomes, polymeric and solid lipid NPs (SLNs), solid lipid carriers, liquid crystals (LCs), microemulsions (MEs), and hydrogels. Different types of nanoparticles have been utilized for targeted delivery to the brain including polymeric nanoparticles, lipid-based nanoparticles, inorganic nanoparticles, and dendrimers (Fig. 13.3). Different

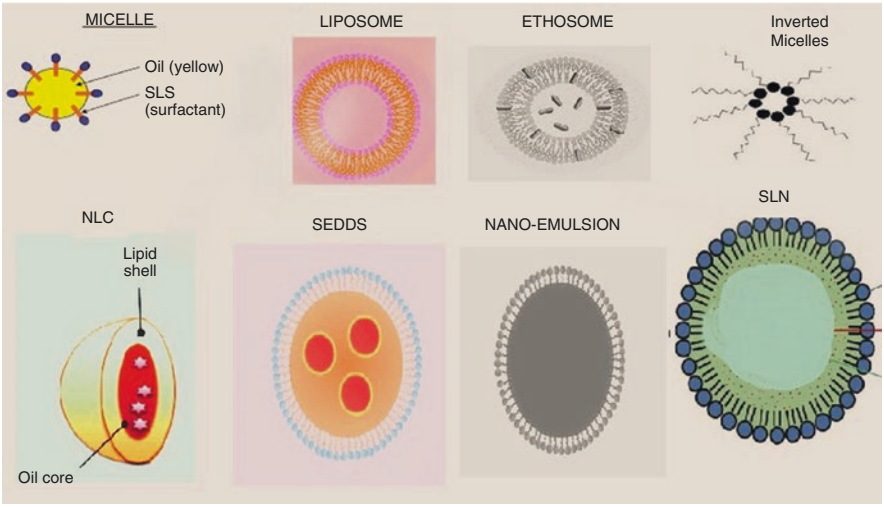

Fig. 13.3 Types of nanolipidic carriers

routes of delivery can be utilized for administering the drug-loaded nanoparticles to the body including parenteral, intranasal, pulmonary, and oral (Saraiva et al. 2016).

Polymeric Nanoparticles

Polymeric nanoparticles constitute the particulate dispersions or are solid particles having a size in range of 1 nm –1000 nm. These particulates may be either in capsular or matrix form depending upon organization of oily or aqueous composition and polymers. Several methods of formulation and development of such NPs have been established like polymerization, ionic gelation and coacervation, emulsification and solvent evaporation, solvent diffusion, nanoprecipitation, spray drying, etc. It has been observed that NPs cross the BBB easily and exhibit increased retention in blood capillaries of the brain which leads to a higher concentration gradient across the endothelial cell layer and thus enhanced delivery of drug to the brain. Further it has been investigated in several experiments that usage of surfactant may solubilize the lipids of the endothelial cell membrane and enhance drug permeability across the BBB. Other fabrication methods including coating NPs with polyethylene glycol (PEG) polymers, or antibodies or mucoadhesive polymers, can increase the retention time of NPs when administered via nasal route (Masserini 2013).

Solid Lipid Nanoparticles (SLN)

SLNs are specifically solid carriers comprising solid lipid core matrix of triglycerides (e.g., tristearin), diglycerides (e.g., glyceryl behenate), monoglycerides (e.g., glycerol monostearate), fatty acids (e.g., stearic acid), steroids (e.g., cholesterol), or waxes (e.g., cetyl palmitate). Generally production of these particles requires stabilization by surfactants to prevent particle agglomeration. Recently a new category of NPs has been developed utilizing a blend of solid lipids in combination with

liquid lipids, namely, nanostructured lipid carriers (NLCs). The primary composition of SLNs or NLCs comprises lipids, emulsifier, and water or solvent and different methods of their preparation are high-pressure homogenization, ultrasonication, high-shear techniques, solvent evaporation, solvent emulsification-diffusion, supercritical fluid method, spray drying, double emulsion and precipitation technique, etc. It has been investigated that these carriers are efficient enough to penetrate and cross BBB easily upon administering through nasal route. Further modifications like use of cationic lipids or coating with surfactants can improve mucoadhesion and thus efficient drug delivery. The advantages that SLN/NLCs offer over polymeric nanoparticles include low cost, easy fabrication, biocompatibility, and high encapsulation efficiency for both lipophilic and hydrophilic drugs (Sonvico et al. 2018).

Recently, Jojo et al. (2019) have evaluated pioglitazone, an antidiabetic drug, for its potential activity in treatment of AD. They optimized the formulation of pioglitazone in the form of nanolipid carriers (NLCs) to target the brain via intranasal route. The in vitro drug release of NLCs was reported to show a sustained release pattern and it was also claimed for improved nasal permeability ex vivo. The toxicity studies conducted confirmed the safety of formulation for the in vivo administration.

In a recent research by Soroor et al. (2019), SLNs and NLCs of curcumin were formulated and targeted to the brain for free radical scavenging in AD. They reported that antioxidant property of curcumin can significantly alter the oxidative stress in AD; however, its targeting to the brain and bioavailability is a big challenge. It was concluded that NLCs and SLNs prepared were capable of crossing BBB. Further, they claimed that upon i.v. administration of free curcumin, SLNs and NLCs, the NLCs were found to be the most bioavailable. The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging study indicated that preparation processes do not have any significant effect on the antioxidant activity of curcumin.

In 2018, Vakilinezhad and coworkers studied the role of nicotinamide in halting AD progression. They prepared nicotinamide-loaded solid lipid nanoparticles (SLNs) functionalized with polysorbate 80 (S80) or phosphatidylserine (PS) or phosphatidic acid (PA). Further the SLNs were evaluated for cytotoxicity, biodistribution, and in vivo effectiveness through the different routes of administration. Also the spatial memory test, histopathology, and biochemical tests were conducted which confirmed effectiveness of PS-functionalized SLNs in improving the cognition, preserving the neuronal cells, and reducing tau hyperphosphorylation in a rat model of Alzheimer's disease.

Liposome

Liposomes are vesicular DDS comprising of one or more phospholipid bilayers over aqueous core and are capable of carrying both lipophilic and hydrophilic drugs. Different methods utilized in preparation of liposomes are sonication, extrusion, high-pressure homogenization and reverse-phase evaporation, etc. Depending upon the number of lipid layers over aqueous core, liposomes may be unilamellar or multilamellar. Further, liposomes can also be of different types according to their fabrication methods and materials used in fabrication, e.g., niosomes, transfersomes,

ethosomes, and phytosomes. Several strategies have been utilized for efficient targeting of liposomes across the BBB including coating and conjugation with certain chemicals or biomolecules. This is also termed as functionalization of liposomes that leads to enhanced uptake and permeability of these carriers across various barrier models. In vivo uptake studies have also been performed in animal models of AD to demonstrate the effectiveness of these nanosystems.

Nowadays health supplements and natural products are emerging as one of the efficient strategies in modulation of therapy of AD. Recently, Yaseen et al. (2018) evaluated the protective effect of nano-wheat germ (NWG) and nano-rice bran (NRB) in AD. The study was conducted in rats having dyslipidemia. Various parameters like lipid profile, level of butyrylcholinesterase (BChE), brain oxidative stress, and inflammatory biomarkers were assessed. The results of biochemical and nutritional parameters reported significant improvement in oxidative stress management in AD.

According to another study, Loureiro et al. (2017) reported that various natural compounds like resveratrol possess potential neuroprotective characteristics that can be utilized in treatment of AD. Evaluation of grape skin seed extracts was done for resveratrol content and tested for its activity for inhibition of Aβ aggregation in the brain. To avoid metabolism and increase bioavailability of resveratrol in the brain, solid lipid nanoparticles (SLNs) functionalized with an anti-transferrin receptor monoclonal antibody (OX26 mAb) were prepared. The results demonstrated active targeting of brain by the fabricated SLNs with massive improvement in AD progression. Some of the recent research works utilizing lipid-based nano-carriers for brain-targeted delivery of anti-Alzheimer drugs are summarized in Table 13.1.

13.5 Role of BBB in CNS Targeting

The hindrance in effective treatment of AD is not just because of the lack of efficacy of drugs but also due to their inability to cross the BBB. Therefore, in order to target delivery of drugs to the brain, it is very important to learn about BBB.

The BBB acts as a mediator between the CNS and the peripheral blood circulation. It is a highly selective physical as well as chemical barrier whose function is to prevent the entry of unwanted foreign molecules and pathogens into the brain while allowing the influx of necessary nutrients, signaling molecules, and immune cells into the brain. BBB is formed by endothelial cells on the blood side and astrocytes and pericytes on the brain side (Chen and Liu 2012). The endothelial cells of the BBB possess an increased number of mitochondria and they form tight junctions (TJs) in association with tight junction proteins such as occludins, claudins, and junctional adhesion proteins which itself are induced by zonula occludens proteins (ZO-1/2/3) and cingulin (Pardridge 2003). The high number of mitochondria in endothelial cells provides high resistance of 1000 Ω/cm^2 which helps in transcytosis (Abbott et al. 2006; Jain 2012) where highly lipo-

Table 13.1 Recent developments in nanotherapeutics in treatment of Alzheimer's disease

S. No	Year	Technology (nanosystem)	Drug/active ingredient	Animal model/cell line	Application/advancement/findings	Reference
1.	2016	Solid lipid nanoparticles (SLNs)	Resveratrol-loaded SLNs, functionalized with apolipoprotein E	Immortalized human cerebral microvascular endothelial cells (hCMEC/D3)	Promising brain targeting of resveratrol-loaded SLNs functionalized with apolipoprotein E Least degradation in the bloodstream No toxicity up to 50 Mm size and high permeability (1.8-fold)	Neves et al. (2016)
2.	2016	Solid lipid nanoparticles (SLNs)	Astaxanthin-1	Pheochromocytoma-12 cell line	Radiolabeled nanoparticles were found to be 96–98% stable even after 48 h of labeling in phosphate-buffered saline (pH 7.4) Comparative biodistribution data indicated higher drug concentration in the brain upon intranasal administration of 99 mtc labeled astaxanthin solid lipid nanoparticles as compared to their delivery via intravenous route Studies on the pheochromocytoma-12 cell line demonstrated the antioxidant potential of astaxanthin solid lipid nanoparticles against H2O2-induced toxicity (oxidative stress)	Chandra Bhatt et al. (2016)
4.	2016	Nanoparticles and solid lipid nanoparticles (SLNs)	Tarenflurbil	Male Sprague Dawley (SD) rats	Comparative study of tarenflurbil (TFB)-loaded poly(lactide-co-glycolide) nanoparticles (TFB-NPs) and solid lipid nanoparticles (TFB-SLNs) were done to evaluate their brain targeting efficiency through intranasal route Brain targeting efficiency was determined in terms of %drug targeting efficiency (%DTE) and drug transport percentage (DTP). The higher %DTE (287.24) and DTP (65.18) were observed for TFB-NPs followed by tfbSLNs (%DTE, 183.15, and DTP, 45.41)	Muntimadugu et al. (2016)

5.	2016	Solid lipid nanoparticles (SLNs)	Chrysin (CN)	Rats with amyloid-β_{25-35}-induced oxidative stress in their hippocampal region	Chrysin SLNs (CN-SLNs) were prepared and investigated for their therapeutic role in neuronal damage upon administration of Aβ25–35 All the antioxidant enzymes and nonantioxidant enzyme in hippocampus were reduced significantly ($P < 0.01$) in the Aβ25–35-injected group, whereas lipid peroxidation and acetylcholine esterase were increased significantly ($P < 0.01$) Claimed for better therapeutic efficacy of CN-SLNs at lower dose as well as high bioavailability	Vedagiri and Thangarajan (2016)
6.	2016	Nanostructured lipid carriers (NLC)	Temozolomide	Mouse/mice injected with TMZ-dispersion (i.v.) and treated with TMZ-NLCs (intranasal)	Prepared a range of TMZ-NLC formulations having sizes in the nanometer range, with high drug loading and prolonged drug release In vivo studies in mice showed that the brain/blood ratio of TMZ-NLC was significantly high, also confirmed by scintigraphy images of mouse brain The AUC ratio of TMZ-nlct to TMZ-disp in the brain was the highest among the organs	Khan et al. (2016)
3.	2015	Solid lipid nanoparticles (SLNs)	Galantamine hydrobromide	Cognitive deficit rats	The SLNs formed were of nanocolloidal range (lower than 100 nm) having drug entrapment up to 83.42 ± 0.63%. And more than 90% drug release in vitro within 24 h In vivo evaluations demonstrated significant memory restoration capability in cognitive deficit rats in comparison with naive drug The developed carriers offered approximately twice bioavailability to that of plain drug	Misra et al. (2015)

(continued)

Table 13.1 (continued)

S. No	Year	Technology (nanosystem)	Drug/active ingredient	Animal model/cell line	Application/advancement/findings	Reference
8.	2013	Nanocapsules	Resveratrol	Male adult Wistar rats (300–350 g) with impaired cognitive functions induced by i.c.v. injection of Aβ1–42	Comparative analysis of neuroprotection provided by free resveratrol versus resveratrol-loaded lipid-core nanocapsule treatment against intracerebroventricular injection of Aβ1–42 in rats Claimed for robust increase of resveratrol concentration in the brain tissue by lipid-core nanocapsules	Frozza et al. (2013)
9.	2013	Liposomes	Rivastigmine	Male albino rats of Wistar strain weighing 260 ± 20 g having deterioration of spatial memory induced by AlCl3	Comparative analysis of rivastigmine liposomes (RLs) w.r.t rivastigmine solution (RS) against aluminum chloride (AlCl3)-induced Alzheimer's model Both RLs and RS improved the deterioration of spatial memory induced by AlCl3, with RLs having a superior effect The profound therapeutic effect of RLs over RS was evidenced by nearly preventing amyloid plaque formation	Ismail et al. (2013)

7.	2012	Nanocapsules	Indomethacin	Male adult Wistar rats (280–330 g) with impaired cognitive functions induced by i.c.v. injection of Aβ1–42	Investigated the potential protective effect of indomethacin-loaded lipid-core nanocapsules (indoh-lncs) against cell damage and neuroinflammation induced by amyloid beta (Aβ)1–42 in AD models Results showed that indoh-lncs attenuated Aβ-induced cell death and blocked the neuroinflammation triggered by Aβ1–42 in organotypic hippocampal cultures Also indoh-LNC treatment led to the increase in interleukin-10 release and decrease glial activation and c-jun N-terminal kinase phosphorylation	Bernardi et al. (2012)
10.	2009	Solid lipid nanoparticles (SLNS)	Ferulic acid	LAN5 cell line	Preparation of SLNS of ferulic acid (FA), a phenolic compound with a significant antioxidant activity in Alzheimer's disease Cells treated with FA-loaded SLN showed a higher reduced reactive oxygen species production than cells treated with free FA	Bondì et al. (2009)

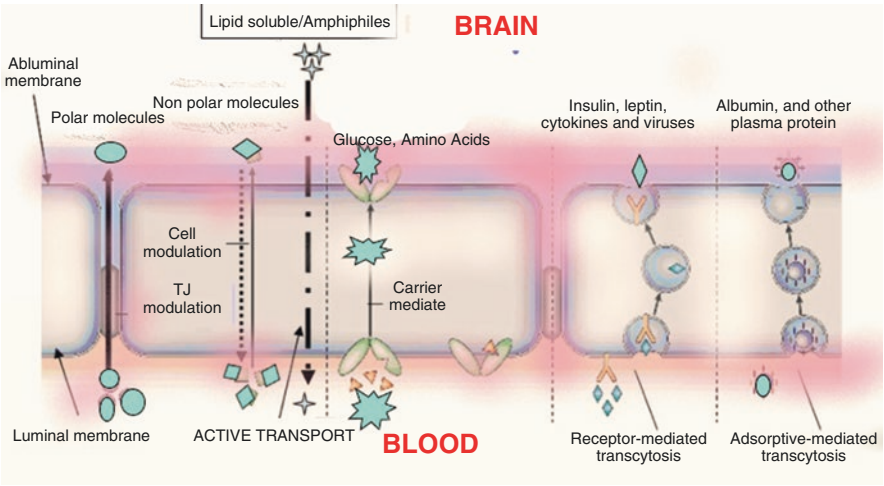

Fig. 13.4 Transport mechanism across blood-brain barrier

philic molecules with a molecular weight <600 g/mol and oxygen and carbon dioxide can easily pass through by passive diffusion, and brain nutrients (i.e., glucose, amino acids) that are highly hydrophobic pass through special transporter proteins actively, while certain larger molecules (i.e., insulin, iron transferrin) tend to pass through receptor-mediated transportation. The different mechanisms of transport by which endogenous and exogenous molecules can travel across BBB are illustrated in Fig. 13.4.

Endothelial cells, astrocytes, and pericytes work together to maintain the integrity of BBB. Astrocytes release some factors, such as transforming growth factor-β (TGF-β) and interleukin-6 (IL-6), which enhance expression of cell signaling proteins such as P-glycoprotein and many others on endothelium (Abbott et al. 2006). Pericytes are involved in endothelial cell growth regulation. Pericytes and endothelial cells are covered by the basement membrane which not only provides mechanical support but also helps in communication of endothelial cells with parenchyma (Dohgu et al. 2005). Endothelial cells also contain cytochrome P-450 system, monoamine oxidase, and other enzymes which prevent the entry of drugs and toxins due to their metabolization (Rubin and Staddon 1999). Besides this, some efflux transporters like P-glycoprotein (P-gp), multidrug resistance protein (MRP), and receptor for advanced glycation end products (RAGE) are also expressed at endothelium surface which actively transports unwanted materials out (Cirrito et al. 2005), while transporter proteins like glucose carrier (GLUT1) and amino acid carrier (LAT1) transport nutrients to the brain. Receptor proteins like insulin receptors, transferrin receptors (TfR), and others present on the blood side of the brain influx larger molecules (mostly peptides, proteins, and lipids) into the brain (Mehta et al. 2013).

13.5.1 How BBB Breakdown Affects Drug Delivery

In AD animal models, BBB dysfunction has been associated with decreased activity of P-gp transporter. As a result, P-gp, which is involved in Aβ efflux under healthy conditions, starts accumulating Aβ in the brain (Chiu et al. 2015). BBB disruption does not increase permeability of drugs across BBB, as drugs can only cross the BBB if the blood vessels are healthy and there is adequate blood flow with recruitment of solute carrier-mediated transport (CMT) and receptor-mediated transcytosis (RMT) systems to facilitate drug delivery. It has been observed that, in the regions of pathological BBB disruption, functional and structural changes in the blood vessels such as perivascular accumulation of blood-derived fibrinogen, thrombin, albumin, immunoglobulin G (IgG), pericyte and endothelial degeneration, RBC extravasations, reduced expression of tight junctions at the BBB, increased endothelial bulk flow transcytosis, disrupted BBB transporter expression, inflammation and immune responses, occur. All of these changes prevent the entry of therapeutic agents to the brain (Chiu et al. 2015; Sweeney 2018). The disrupted BBB enables blood-derived debris and cells to accumulate in enlarged perivascular spaces. These accumulations prevent the normal distribution of molecules throughout the CNS and interrupt the regional formation of interstitial fluid (ISF) and ISF flow, which prevent therapeutic antibodies, proteins, peptides, gene medicine, and other drugs from effectively reaching their neuronal targets. Besides this function of CMT and RMT systems is also decreased which further complicates the therapeutic drug delivery process (Nelson et al. 2016). Therefore, for the successful delivery of therapeutic agents into the brain of AD patients, healthy blood vessels are needed.

13.5.2 Transport Mechanism of Nanolipidic Carriers Across BBB

Generally, most of the lipid-based nanocarriers are grouped into two major classes: nanoparticles (NPs) and liposomes (LPs). Though LPs are well known for their constitution and drug-loading efficiency, still they are not capable of effectively passing through a healthy BBB. However it has been observed that upon functionalization or surface modification with polymers, polysaccharides, peptides, or antibodies, brain targeting can be achieved successfully.

Torre and Ceña (2018) reported PEGylated liposomes are prevented from being eliminated by the immune system and have a controlled bio-distribution. Furthermore, they revealed that positively charged LPs exhibit improved interactions with the cell membrane and hence promote enhanced uptake and therefore are well suited for delivering ionic drugs and genetic materials.

Holtzman et al. (2012) worked upon apolipoprotein E (ApoE) receptors and designed nanocarriers with surface modified with ApoE which were reported as highly BBB permeable and efficient in the treatment of Alzheimer's disease (AD).

In case of SLNs, the lipids present in the DDS facilitate higher entrapment of lipophilic compounds as well as their passage across BBB with ease. NLCs are a subclass of SLNs characterized with comparative high drug loading and biocompatibility.

13.5.3 Implications for Drug Therapy in AD

Current drugs, AChEIs, used in AD therapy have ease of oral administration but they are less selective and therefore also have action on peripheral tissues leading to side effects. This problem can be solved by designing drug therapies which are able to cross the BBB and deliver drugs directly into the CNS. Nanoparticles (NPs) represent a very promising approach to facilitate BBB crossing and delivering therapeutic compounds into the brain by the use of the mechanisms of transcytosis or, more specifically, a receptor-mediated pathway. The addition of polyethylene glycol (PEG; PEGylation) to NPs is the FDA-approved method which is also used now. Nanoparticle preparation of existing drugs are being designed such as liposome preparation of rivastigmine and galantamine which allow their intranasal administration with diminished adverse effects. SLN preparations of donepezil and galantamine improved cognition compared to free drug. Polymeric nanoparticle preparation of rivastigmine and galantamine with chitosan resulted in improved bioavailability and improved uptake of both the drugs in the brain after intranasal administration. Besides this, nanoliposomic preparation of herbal drugs such as curcumin retarded Aβ aggregation. Solid lipid nanoparticle preparation of resveratrol prevented Aβ peptide fibrillation (Sweeney 2018).

13.6 Conclusion and Future Prospects

According to recent studies it has been estimated that more than 12 million people worldwide are AD patients which is going to increase manifolds in upcoming years. In this scenario, scientists are working progressively in field of nanotechnology for designing and development of nanolipidic carriers which are potential vehicles for targeting CNS-related diseases. Among various routes of administration, nasal route has shown promising results to target the brain, specifically in treatment of AD. Intranasal delivery has emerged as an alternative route to oral and parenteral administration as it is a noninvasive method for direct nose to brain drug delivery bypassing the BBB. Delivery to the brain via this route occurs through the olfactory region and respiratory epithelium since the olfactory nerve cells and trigeminal nerves are in direct contact with both the nasal cavity and the CNS. Though nasal route is the best approach, however, oral, dermal, and intravenous routes can also be evaluated for administration of such nanolipidic carriers to target to the brain. It has been recorded that nanotechnology-based products are increasing in market day by

day but still clinical trials are needed to evaluate their safety and efficacy in humans. Nanolipidic carriers have the potential to emerge as the most sought-after drug delivery vehicle in the near future for targeted drug delivery in AD that could lead to improved therapeutic outcomes with reduced costs.

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Chapter 14
Curcumin and Its Nanoformulations as Therapeutic for Alzheimer's Disease

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Abstract Since decades curcumin has been known for its pleiotropic nature and for its biological effects which broadly include its antioxidant, anti-inflammatory, and anticancer potential. Plethora of published articles are a proof of pluripotent effect of curcumin against various neurodegenerative disorders including Alzheimer's disease. Mechanistically, naive curcumin inhibits the formation of amyloid-β plaques, attenuates the hyperphosphorylation of tau and enhances its clearance, binds copper, lowers the cholesterol level, modifies microglial activity, inhibits acetyl cholinesterase, mediates the insulin signaling pathway, and is reported to be a very effective antioxidant. However, its usefulness as a therapeutic agent is hindered by its compromised bioavailability due to low aqueous solubility (11 ng/mL) and low permeability (log 3.28). Nano-delivery systems like liposomes, polymeric nanoparticles, micelles, conjugates, peptide carriers, cyclodextrins, solid dispersions, lipidic nanoparticles, and emulsions have been extensively explored for enhancing the overall bioavailability of curcumin. This book chapter has been written to describe scope of using curcumin and its nanoformulation(s) as therapeutics for Alzheimer's disease. Ongoing clinical trials on curcumin are also covered. Various curcumin-based products currently available in the market or those in the pipeline are also discussed.

Keywords Curcumin · Nanostructured systems · Memory · Cognition · Mechanism · Clinical trials · Pipeline products

14.1 Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease which arises with unnoticeable small deviations in the brain neurons tangled with cognitive function (Martina 2019). Typically, individuals live with Alzheimer's symptoms for years. Over time, symptoms tend to rise and start impeding with individual's

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capability to perform routine everyday activities (Reitz and Mayeux 2014). With progression of the disease to other parts of the brain, the intellectual capability and other body functions of the body start getting affected. Gradually, the patient is restricted to bed and needs perpetual care (Rebecca et al. 2003).

Pathologically, the brain of patient suffering from Alzheimer's disease has neuronal circuits with exaggerated buildup of beta-amyloid (called beta-amyloid plaques) and protein tau (called tau tangles) (Nima et al. 2019). Accumulation of beta-amyloid on the exterior part of neurons may subsidize to cell death by impending communication between neuron while protein tau deposition on inner part of neurons blocks the transport of nutrients and other essential molecules (Alzheimer's Association 2019). Other factors which may further worsen the condition include inflammation, oxidative stress, and atrophy.

Alzheimer's Association published a report on "Alzheimer's disease facts and figures 2019" which showed that total of 5.8 million Americans were affected with Alzheimer's dementia.

Alzheimer's disease facts and figures 2018 recounted that total 5.7 million people are living with AD across the globe. It was reported that one out of 10 people at age 65 and older is affected with Alzheimer's disease. Further two-third ratios of American population with Alzheimer's are women as shown in Fig. 14.1. Also the older African-Americans are at double danger of having Alzheimer's or other dementias than older whites. With this, an estimated amount of $277 billion is spent for healthcare, long-term care, and hospital services of AD patients. The latter not only influences the quality of life of the patients but also adds on to a socioeconomic health burden.

Brasure et al. (2018) performed a holocultural study of India and the United States and revealed that Indian inhabitants aged 70–79 had a 4.4 times lesser incidence of AD compared to the similar inhabitants in the United States. American Association 2019 report has very recently stated that the individuals (at phase 65 and above age) with Alzheimer's disease are expected to grow to 13.8 million in

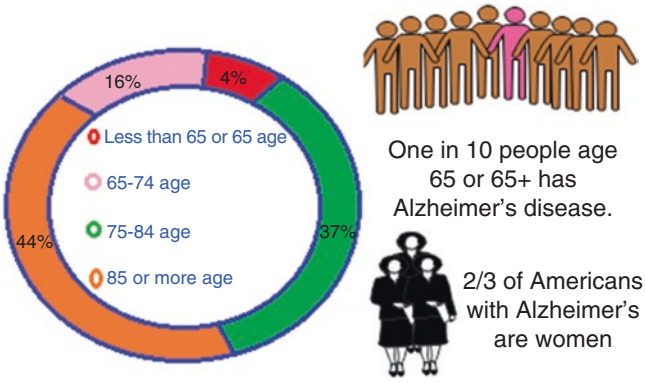

Fig. 14.1 Prevalence of Alzheimer's disease throughout the world

spite of new medicinal revolutions to preclude, slow, or cure Alzheimer's disease by 2050.

Available treatment therapies though provide a symptomatic relief, however, are associated with side effects on long-term use and a high degree of recurrence. The irony is that though large numbers of nanotechnological breakthroughs are coming up for AD, however none has reached the end user. This book chapter is written with an aim to present an overview of curcumin-based nanoformulations which have been developed and have a futuristic scope for research exploration.

14.2 Etiology of Alzheimer's Disease

Since the last decade, tremendous progress has been through in defining the multifactorial etiology of AD. Known characteristic risk findings for AD comprise chromosome mutation, neurodegeneration, senile plaques, loss of synaptic connections, vascular dementia, beta-amyloid, and genetic factor as shown in Fig. 14.2. Additional possible risk features are level of education, female sex, the past of head injury, exposure to heavy metals and toxins, and positive family history (Mendiola et al. 2016). Mostly people with minor cognitive impairment are at greater risk for developing Alzheimer's disease. The leading indications of Alzheimer's differ in different individuals; it may diverge from weakening in some features of cognitive memory, i.e., difficulty in word spatial issues in addition to impaired judgment ability.

Research on various biomarkers in cerebrospinal fluid and blood give a clear evidence of significant changes in AD brain, which aid in early treatment therapy, thus increasing the chances of timely treatment (Alain et al. 2017).

14.3 Treatment Options for Alzheimer's Disease

Though numerous symptomatic treatments exist for this Alzheimer's disease, none of them provides a complete cure. USFDA (Food and Drug Administration) approved several drugs belonging to class cholinesterase inhibitors (i.e., rivastigmine, galantamine, donepezil) (Alva and Cummings 2008) and NMDA antagonist (memantine). Some are listed in Table 14.1. Different combinations of memantine with donepezil and tacrine are presently available in the market (Konstantina and Sokratis 2013). The therapeutic regimen and their efficacy is highly variable with chances of side effects and recurrence, once the medication is stopped.

Curiosity in the use of nutraceuticals and phytochemicals is emerging nowadays. Investigators reported that about 25% of existing drugs are prepared from herbal medicines. Numerous phytochemicals known to possess anti-Alzheimer's properties include *Ginkgo biloba*, lignans, flavonoids, tannins, polyphenols, triterpenes, sterols, and alkaloids. Numerous studies have corroborated that curcumin is safe

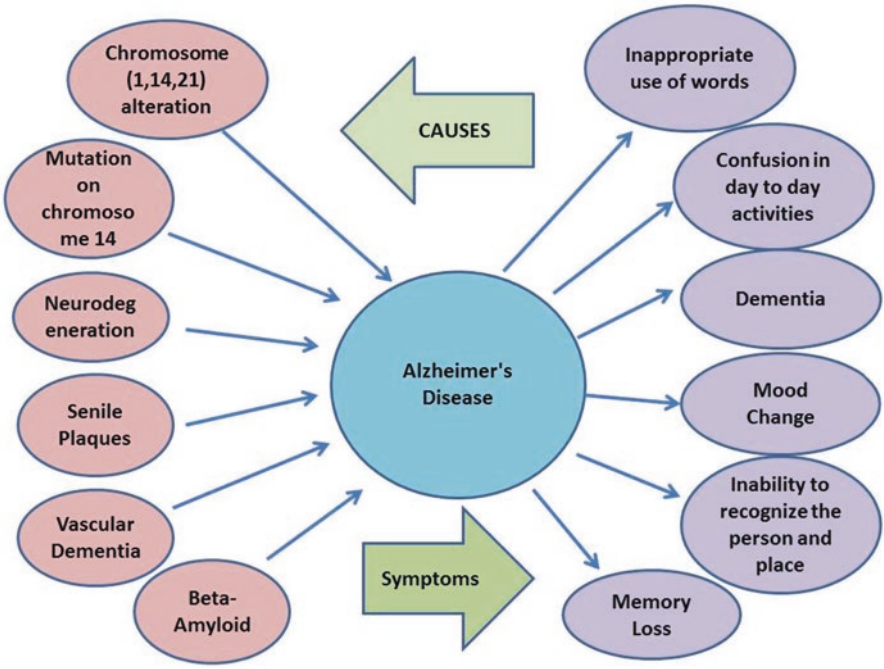

Fig. 14.2 Causes and symptoms of Alzheimer's disease

and effective in humans (Mecocci et al. 2014). Curcumin possesses diverse pharmacological functions such as anti-inflammatory, anti-amyloidogenic, anticholinesterase, and antioxidant activities (Bushra et al. 2018).

14.4 Why Curcumin for Alzheimer's Disease?

Curcumin, well-known as haldi, is obtained from *Curcuma longa*, a rhizomatous plant of Zingiberaceae family. Since decades, curcumin is used as a food additive and currently is used in some forms comprising capsules, tablets, ointments, soaps, and cosmetics for treatment of various diseases (Gupta et al. 2013). The major benefit of curcumin is that even in larger doses it is known to precipitate minimal side effects. Curcuminoids are approved by the US Food and Drug Administration (FDA) as "generally recognized as safe" (GRAS) (Susan et al. 2017) components. This phytochemical compound possesses low molecular weight (368.38 g/mol) with two aromatic o-methoxy phenolic groups which are interconnected to alpha, beta-unsaturated carbonyl groups and diketones through a seven-carbon chain (Shome et al. 2016). Indira Priyadarsini (2013) summarized chemical and structural features influencing the biological activity of curcumin. They stated that o-methoxyphenol group and methylenic hydrogen are apt for antioxidant potential of curcumin (as curcumin

Table 14.1 Marketed products for Alzheimer's disease

Class of drug	Example	Mechanism of action	Side effects	Reference
Cholinesterase inhibitors	• Donepezil (5 mg, 10 mg tablet) • Rivastigmine (Exelon) (4.5 mg, 6 mg, 2 mg/mL tablet, solution) • Galantamine (Razadyne) (4 mg, 8 mg, 12 mg tablet)	These drugs work by slowing the chemical messenger (Ach) in the brain that is important for learning and memory Maintains Ach levels in brain	Nausea, vomiting, diarrhea, muscle cramps, weight loss, headache	David (2014)
NMDA (N-methyl-D-aspartate) antagonists	• Memantine (Namenda) (5 mg,10 mg tablet)	Regulates the activities of different chemical messengers (glutamate) in the brain that is also import for learning and memory	Tiredness, body aches, joint pain, dizziness, diarrhea, constipation	David et al. (2012)
Antioxidant	• Vitamin E (Aqua-E, Aquasol E)	Scavenges free radicals, influences the expression of genes involved in apoptosis, causes dopaminergic neurotransmission, helps in clearance of Aβ	Increases the chances of having a serious stroke called hemorrhagic stroke (at dose 300–800 IU per day)	Agnese et al. (2017)
Peroxisome proliferator-activated receptor γ (PPARγ) agonist	• Pioglitazone (15–30 mg)	Affects gene transcription and reduces inflammation	Chest pain, decreased urine output, dilated neck veins, extreme fatigue, irregular breathing	Galimberti and Scarpini (2017)
Gamma secretase inhibitors (phase III trials)	• Semagacestat (100 mg,140 mg, 280 mg)	γ-Secretase inhibitor		Doody et al. (2013)

hydrogen atom interacts with reactive oxygen species) (Indira Priyadarsini 2013). Curcumin intermixes with a number of biomolecules through non-covalent and covalent binding (Anand et al. 2018). Aromatic and tautomeric configurations of curcumin together with the elasticity of the linker group are responsible for the non-covalent interactions (Reinke and Gestwicki 2007). The alpha, beta-unsaturated diketone moiety covalently relates with protein thiols, thru Michael reaction. The diketo group forms chelates with transition metals; hence reducing the metal persuaded toxicity and certain metal complexes show improved antioxidant activity as enzyme mimics. New analogues with enhanced activity are being developed using modifications on specific functional groups of curcumin (Chen et al. 2011). Orlando et al. (2012) stated that alteration of hydroxyl groups in the aromatic rings of curcumin

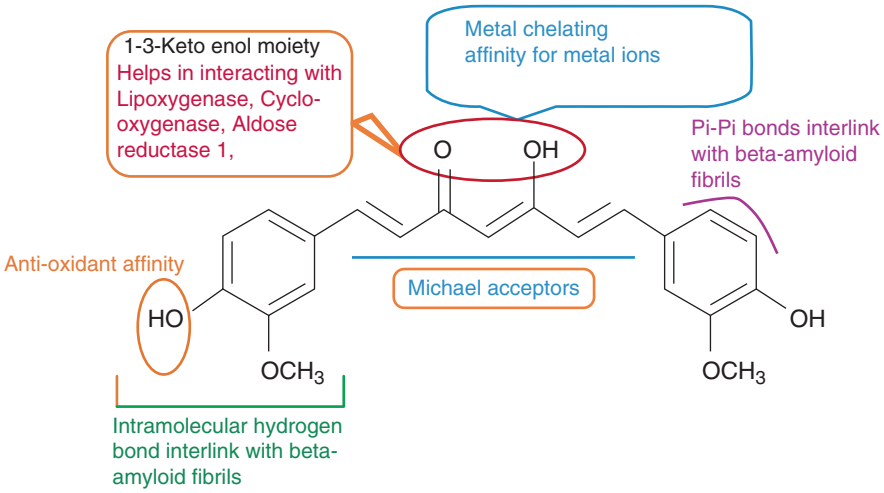

Fig. 14.3 Structure of curcumin indicating functional groups with potential for the treatment of Alzheimer's disease

abolished Aβ inhibitory effect, hence proposing that aromatic substitution accomplished with forming hydrogen interactions is critical to maintain the binding activity of curcumin ligands to Aβ. In addition, the optimal length of linker region between 8 and 16 A° is needed for the binding affinity of curcumin toward Aβ (Orlando et al. 2012) as shown in Fig. 14.3.

Furthermore, the polar groups offer a site for deprotonation and ensuing binding position for Aβ oligomers. Huge database supports the therapeutic activity of curcumin in AD (Cornago et al. 2008). Its lipophilic nature and neutral hydrophobic carbon bridge between polar enolic and phenolic groups makes it feasible to cross the blood-brain barriers (Kavirayani 2014).

Within two decades, nearly 3000 research articles have been published which describe the therapeutic efficacy of turmeric. However, curcumin is widely examined by different authors in treatments also targeting the Aβ pathway and is being tested under advanced clinical trials. They also may be used with aim of reducing behavioral symptoms such as depression, apathy, wandering, sleeping troubles, nervousness, and aggression (Prasad et al. 2014).

14.5 Unique Diagnostic Capability of Curcumin

Major biomarkers which include amyloid-β and highly phosphorylated tau protein are used for the purpose of diagnosis of AD. However, curcumin properties such as lipophilicity, natural fluorescence, ability to cross blood-brain barrier, and high binding affinity to amyloid-β make it as early diagnostic probe/plaque labelling fluorochrome (Chen et al. 2018). Wang et al. (2006) identified curcumin to fluoresce

yellow/green under a violet/blue (436 nm) light. In recent times, these native fluorescent qualities of curcumin, which absorbs light at about 420 nm and emits fluorescence at around 530 nm in aqueous solutions, have been extensively explored for diagnostic purpose. During the last 20 years, extensive research has been performed to develop curcumin probes for targeting Aβ with existing imaging modalities, with positron emission tomography (PET), two-photon microscopy, magnetic resonance imaging (MRI), and near-infrared fluorescence (NIRF) (Tu et al. 2015).

14.6 Insight into the Anti-Alzheimer's Mechanisms of Curcumin

The progression of AD starts with degradation of nerve cells and is believed to include certain properties such as inflammatory reactions, oxidative damage, and, especially, the growth of beta-amyloid plaques and metal toxicity. There have been abundant studies on properties of curcumin on AD. Curcumin has exposed to possess anti-Alzheimer's effects by overwhelming oxidative damage, inflammation, cognitive deficits, and amyloid accumulation (Mishra and Palanivelu 2008). The details of the mechanisms are discussed below (Fig. 14.4).

14.6.1 Based on Its Anti-inflammatory Activity

One most vital pathogenic event in Alzheimer's disease is the prolonged inflammation of nerve cells. A number of inflammatory reactions like microgliosis and astrocytosis lead to the accumulation of amyloid-β (Aβ) peptide (Giri et al. 2004). Researchers have explored the experimental and pharmacological trials of curcumin to determine its efficacy as an anti-inflammatory agent (Pendurthi and Rao 2000).

Curcumin shows its anti-inflammatory activity by inhibiting lipoxygenase post binding with it or to phosphatidylcholine micelles (Shrikant and Kalpana 2008). The in vitro and in vivo experimentation discovered that curcumin blocks aggregation and fibril formation by directly binding to small β-amyloid species. In vitro studies have shown that curcumin also inhibits amyloid plaque-mediated transcription related to cytokine regulation (John et al. 2005) and suppresses inducible nitric oxide synthase (iNOS) in activated macrophage processes that finally promote inflammation. As oxidant-like lipid peroxides cause inflammation, curcumin's antioxidant properties serve to decrease inflammation.

María et al. (2018) reviewed that curcumin blocks interleukin-1 (IL-1) signaling by inhibiting the recruitment of the IL-1 receptor and suppresses IL-1β secretion through inhibition of the Nod-like receptor protein 3 (NLRP3) inflammasome (María et al. 2018).

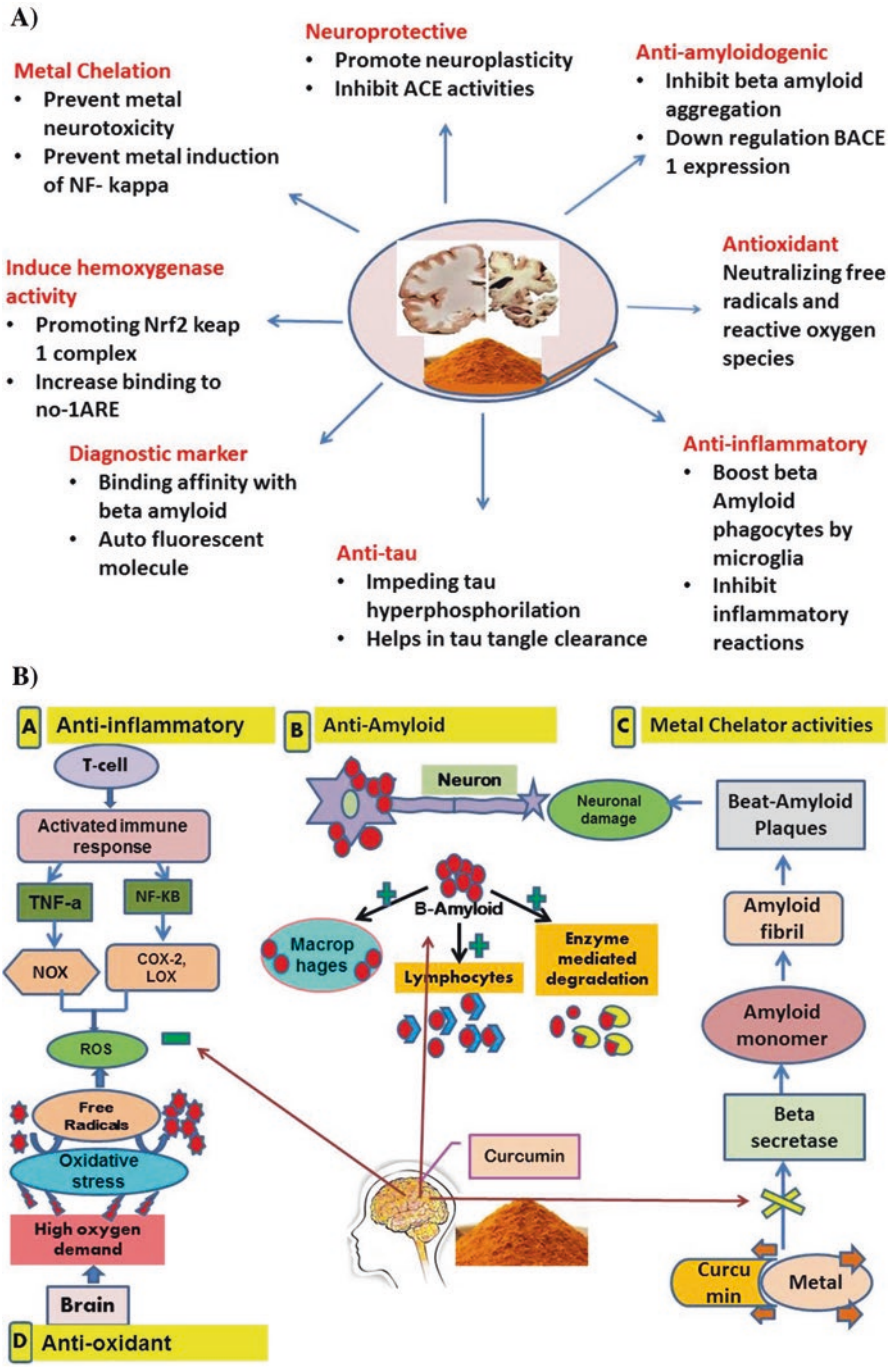

Fig. 14.4 Purported mechanism of curcumin for treatment of Alzheimer's disease. (**a**) General mechanism. (**b**) Steps involved in specific mechanistic pathways

In another study by Panahi et al. (2016), the effect of curcumin supplementation on serum cytokine concentrations in subjects with metabolic syndrome (MetS) was reported. 117 individuals who met the enclosed measures were arbitrarily allocated with daily dose of 1 g/day of curcumin ($n = 58$) or a matched placebo ($n = 59$) for a period of 8 weeks. Their comparative results showed significant greater reductions in serum concentrations of TNF-α, IL-6, TGF-β, and MCP-1 post curcumin administration versus placebo group ($p < 0.001$) (Panahi et al. 2016).

Lim et al. (2011) demonstrated that curcumin decreases Aβ-ROS-related inflammation and beta-amyloid burden in amyloid precursor protein (APP) transgenic mice (with Swedish mutation). Both low and high doses of curcumin significantly decreased IL1β level. IL1β increases the production and processing of APP, thus favoring deposition of amyloid-β (Lim et al. 2011). A low dose of curcumin significantly decreases activated glial marker, glial fibrillary acidic protein (GFAP); thus it ameliorates glial-mediated inflammation. Curcumin has been reported to downregulate several neuroinflammatory marker proteins, including nuclear factor kappa beta (NF-κB). Likewise, curcumin can also inhibit the proinflammatory pathways by activating peroxisome proliferator-activated receptor gamma (PPARγ) (Buhrmann et al. 2011).

14.6.2 Based on Its Anti-oxidative Activity

Oxidative stress is an imbalance between in the redox state, involving the buildup of ROS, or a decrease in defensive mechanism of antioxidant. The brain is mainly sensitive to oxidative stress as it has a high oxygen consumption rate and less enzymatic defense against oxidative stress and is composed of lipids that are easily oxidized (Huang et al. 2016). Enhanced levels of lipid peroxidation, DNA, and protein oxidation products such as 8-HO-guanidine and protein carbonyls lead to increase in oxidative stress in AD (Smith et al. 2007). Under oxidative condition, dityrosine cross-linked dimers are formed when reactive oxygen species (generated from formed Aβ plaques) combine with free radicals. As Aβ-induced oxidative stress in neuronal cells acts as a source of AD pathology, one of the pharmacological approaches for AD is antioxidant therapy (Tsuyoshi et al. 2010). Curcumin directly scavenges free radicals and indirectly upregulates the cytoprotective responses (Gibellini et al. 2015). Curcumin was found to inhibit Aβ-induced mitochondria-mediated apoptosis through regulation of the B-cell lymphoma 2 (Bcl-2) protein family, a group of proteins involved in apoptosis (Fan et al. 2017). Mythri et al. (2007) demonstrated that curcumin protects brain mitochondria against various oxidative stresses including reactive radicals of nitric oxide with superoxide (called peroxynitrite). It acts by direct detoxification on versatile oxidant that can attack a wide range of cells and in vivo by raising total cellular glutathione levels (Mythri et al. 2007; Shrikant and Kalpana 2008; Kakkar et al. 2011).

14.6.3 Based on Its Anti-amyloid Activity

Since the deposition of Aβ plaques is the characteristic feature of AD, curcumin has been studied for its ability to prevent the formation and accumulation of Aβ. Panchanan et al. (2018) compared the Aβ aggregation inhibition, anti-amyloid, anti-inflammatory responses of curcumin, and solid lipid curcumin particles (SLCP) in both in vitro and in vivo models of AD. Wild-type mice were treated with intraperitoneal injections of curcumin or SLCP (50 mg/kg body weight) for 2 or 5 days. They observed that curcumin, SLCP, was relatively more permeable and effective and produced a larger decrease in Aβ plaque loads in the prefrontal cortex (PFC) and dentate gyrus (DG) of the hippocampus. Correspondingly, relative to curcumin, SLCP produced a larger decrease of pyknotic, or tangle-like, neurons in PFC after 5 days of treatment (Panchanan et al. 2018; Maiti et al. 2018).

In another study Gary et al. (2018) reported the effect of curcumin on memory in non-demented adults using positron emission tomography (PET). A total of 41 subjects of age 51–84 years were treated with curcumin containing 90 mg twice daily ($N = 21$) or placebo ($N = 19$) for 18 months. They concluded that daily oral dose of curcumin may lead to improved memory and attention in non-demented adults. The PET findings suggested that symptom benefits are associated with decrease in amyloid and tau accumulation in brain regions modulating mood and memory (Gary et al. 2018). Veldman et al. (2016) showed that when aged mice with β-amyloid plaque were fed with curcumin, it reduced the amount of plaque deposition (Veldman et al. 2016).

Another study investigated the effects of curcumin derivatives on Aβ aggregation and the cell toxicities of Aβ aggregates (Reddy et al. 2018) They reported that 14 out of 41 compounds showed a significant increase in the densities of the bands of Aβ (1–42) by incubation during the aggregation process relative to those of Aβ (1–42) prepared in the presence of the vehicle control. A significant positive correlation was observed between the cell viability and densities of the bands in the range of 15–20, 20–37, 37–75, and 75–200 kDa in SDS-PAGE. On the basis of these results, they proposed four curcumin derivatives with potential for preventing AD. These curcumin derivatives exhibited high inhibitory effects on Aβ aggregation and induced the formation of lower molecular size Aβ species that have weaker cell toxicity (Daijiro et al. 2015).

In vivo experiment conducted by Douglas et al. (2012) evaluated the activities of turmeric extract HSS-888 using transgenic "Alzheimer" mice (Tg2576) overexpressing Aβ protein. Their results showed that curcumin extract HSS-888 acts by inhibiting or improving plaque burden, tau phosphorylation, and microglial inflammation which finally reduces the neuronal toxicity (Douglas et al. 2012).

Another report by Yang et al. (2005) has shown that low doses of curcumin diminished 40% and 43% levels of beta-amyloid and "plaque burden," respectively, in AD mice in comparison to those that were not treated with curcumin. It was

found that low doses of curcumin when administrated for longer times were more effective than high doses, as at higher concentration, curcumin binds to amyloid beta and blocks its self-assembly. The in vitro study further supports the potential of curcumin to inhibit Aβ aggregation as well as disaggregates to form fibrillar Aβ40 (Yang et al. 2005). Zhang et al. (2010) demonstrated for the first time that administration of 20 μM curcumin caused 40% reduction of Aβ40 and Aβ42 levels in mouse primary neuronal cells.

14.6.4 Based on Cholesterol-Lowering Properties

Cholesterol has a stimulatory effect on β-amyloid (Aβ) deposition by the intracellular accretion of cholesteryl ester (Puglielli et al. 2003). Additionally, curcumin enhances the inflammatory cascade in its reaction with cholesterol. Investigators believe that by inhibiting cholesterol formation and diminishing serum peroxide, curcumin might exert beneficial effects on AD. In one study, administration of 500 mg of curcuminoids a day for 7 days reduced levels of serum cholesterol and lipid peroxides in healthy volunteers (Soni and Kuttan 1992).

Increased concentration of metals, i.e., copper, zinc, and iron in Aβ plaque, has been reported and in vitro studies have confirmed their role in aggregation of Aβ and subsequent ROS production (Puglielli et al. 2003). Baum and Ng (2004) showed that curcumin can bind with copper and iron ions which act as chelators and reduce Aβ plaque and subsequent ROS generation. Furthermore, induction of NF-kB can occur in the presence of copper that can be prevented by the chelation activity of curcumin (Baum and Ng 2004).

14.6.5 Based on Metal Chelator Activities

Oxidative stress because of accumulation of heavy metals, such as aluminum (Al), copper (Cu), cadmium (Cd), iron (Fe), lead (Pb), manganese (Mn), and zinc (Zn), can cause aggregation of proteins and lead to amyloid plaques (Liu et al. 2011). Wanninger et al. (2015) showed that phenolic (OH) groups and one of the active methylene (CH2) groups of curcumin act as ligand for metal chelation. The curcumin-metal complexes also show greater antioxidant properties. Cu-curcumin complexes scavenge ROS more efficiently than curcumin alone. Amalraj et al. (2017) found that Mn-curcumin complex exhibits a more potent neuroprotection than curcumin, as shown in the in vitro and in vivo experiments (Amalraj et al. 2017). Eybl et al. (2004) further supported this activity of curcumin which interacts with cd and pb to prevent neurotoxicity (Eybl et al. 2004).

14.7 Inherent Challenges with Curcumin

Curcumin is a compound with multifactorial activities but due to some challenges its use as therapeutic agent is limited. With a poor aqueous solubility of 3.12 mg/L at 25 °C, 0.0004 mg/mL at pH 7.3 (3×10^{-8} M), it is insoluble in cold water and ether and slightly soluble in hot water (Gera et al. 2017). It is unstable at physiological pH and in acidic and neutral solutions (pH 2.5–7.0) and emits bright yellow hue and turns red at pH above 7 (Lee et al. 2013). Formation of harmful decomposition products is reported under improper storage conditions. Its body clearance rate is so high that only a trace amount of curcumin appears in blood plasma, even if high doses are administered orally, and is mostly excreted along with the feces and urine (Gupta et al. 2013). When administered orally, 75% of curcumin is excreted in the feces while only traces appear in the urine. It easily gets degraded if exposed to alkaline conditions (pH $\geq$ 7.0) or crystallizes out of aqueous acidic solutions (pH < 7) or else chelates with metal ions (Šimeček et al. 2011). It displays extensive intestinal and hepatic metabolism (Pan et al. 1999; Anand et al. 2007; Kakkar et al. 2011).

Therapeutic effects of curcumin in treating Alzheimer's disease (AD) are well documented and gaining importance. It depends extensively on its ability to easily cross the blood-brain barrier (BBB). The efficiency of the medication also depends on the bioavailability achieved at the target location (Prado et al. 2019). Targeting the central nervous system has always been a major challenge due to its poor inaccessibility. The stringent blood-brain barrier severely restricts the delivery of therapeutic molecules to the brain (Fonseca et al. 2015; Kakkar et al. 2018).

Curcumin can bind to Aβ plaques and prevent their aggregation (Reinke and Gestwicki 2007). Even at a low concentrations such as 0.1–1 μM, curcumin can inhibit Aβ fibril formation, thus helping in reducing brain amyloid level and plaque burden (Yang et al. 2005).

14.8 Clinical Trials of Curcumin

Various clinical trials are in progress or have been concluded to evaluate the efficacy of curcumin as a therapeutic molecule (http://clinicaltrials.gov) (Table 14.2). Several reports on curcumin are under trials for AD. Human trials need to be conducted to establish their effectiveness in clinical applications as an improved therapeutic modality. It also binds to many types of metals, like iron and copper, and can also act as an iron chelator. It is nontoxic and holds great promise as a therapeutic agent and is in various stages of human clinical trials for a plethora of conditions, such as myeloma, pancreatic cancer, colon cancer, and Alzheimer's disease (Hatcher et al. 2008).

Clinical trials to investigate the clinical benefits of curcumin are still under early phases. Early trials are done emphasizing its safety and pharmacokinetics. Current

Table 14.2 Clinical trials of curcumin molecule for Alzheimer's disease

Trial	Status of the trial	Disease targeted	Objective of the study	Clinical trial identifier[a] or reference
Pharmacokinetics of curcumin in healthy volunteers	Ongoing currently	None	Curcumin pharmacology with piperine or silybin	NCT00181662
Curcumin in patients with mild to moderate Alzheimer's disease	Ongoing currently	Alzheimer's disease	Safety, biodistribution, efficacy	NCT00099710
Pilot study of curcumin and ginkgo for treating Alzheimer's disease	Closed	Alzheimer's disease	Effect on amyloid beta protein, cognitive function	NCT00164749
Effect of curcumin on biomarkers of Alzheimer's pathology in elderly population particularly	Results yet to be published	Alzheimer's disease	Bioavailability, safety, and tolerability	Ringman et al. (2005)

[a]Clinical trial identifier with references are the same and we replacing clinical trial identifier with references

trials also aim to explore its efficacy (Hatcher et al. 2008). Moreover, curcumin availability is less in the brain than other organs (Vareed et al. 2008). Till date, nine human trials of curcumin in interventions of Alzheimer's including its diagnosis, prevention, and therapy have been carried out (Chen et al. 2018). Although benefits of curcumin are being extensively evaluated all across the globe, most of these clinical trials are being carried out in the United States of America. The estimated primary completion time for most of these ongoing trials ranges from about 6 months to nearly 10 years. These trials for various diseases are in different phases and are using curcumin mostly in the form of nanoparticles, capsules, tablets, powder, and solutions (Yallapu et al. 2015). Doses ranging mainly from 0.18 to 8 g/day are being utilized for these clinical trials.

For some disorders, curcumin is also administered in combination with other agents and used for therapies such as chemotherapeutics and as nutraceuticals. These ongoing clinical trials are expected to provide a deep understanding of curcumin's efficacy and its mechanism of action. Common to all of these studies has been the safety, tolerability, and nontoxicity of the administered polyphenol (Kunnumakkara et al. 2017). From the findings till date of the completed trials, it seems that curcumin possesses a significant clinical efficacy. However, this polyphenol is not still approved for human use. Reports of its poor bioavailability and adverse effects reported by some investigators are a major limitation to the therapeutic utility of molecule (Gupta et al. 2013).

In a study by Ravindranath and Chandrasekhara (1980), in which 400 mg curcumin was given by oral administration to rats, 60% of the total dose was absorbed. None was detectable in urine. The urinary excretion of conjugated glucuronides and sulfates considerably increased. Only traces (less than 5 μg/mL) were found in portal blood and negligible quantities present in the liver and kidney (<20 μg/tissue) were observed from 15 min up to 24 h after its administration. At the end of 24 h the

concentration of curcumin remaining in the lower part of the gut that is cecum and large intestine amounted to 38% of the total quantity administered (Ravindranath and Chandrasekhara 1980).

In addition to studying the fate and benefits of curcumin administered in rat models, Shoba et al. (1998) administered 2 g of curcumin powder to fasting stage volunteers to demonstrate levels of low curcumin concentrations in plasma (10 ng/mL). In the same study, simultaneous administration of 20 mg of piperine molecule appeared to increase availability of curcumin in humans by a factor of 2000% (Shoba et al. 1998). Ireson et al. (2001) showed that curcumin undergoes extensive first-pass glucuronidation, thus resulting in rapid removal via bile and urine (Ireson et al. 2001).

A phase 1 clinical study of curcumin by Cheng et al. (2001) confirmed that even with a dose of 8 g/day, the amount found in plasma was still 1.77 μM. Such low concentration may be attributed to its metabolism and rapid degradation at a pH > 7 (Tønnesen and Karlsen 1985; Anand et al. 2007). Pharmacologically active concentrations of curcumin were achieved in colorectal tissue of patients consuming curcumin orally and were achievable in tissues such as the skin and oral mucosa, which were directly exposed to the drug moieties when applied either locally or topically. The effect of curcumin was also studied in patients suffering from rheumatoid arthritis, inflammatory eye diseases, inflammatory bowel disease, hyperlipidemia, and cancers (Hsu and Cheng 2007).

A clinical study conducted by a group of researchers showed that neurodegenerative patients (n = 25) showed no signs of toxicity post administration of 500–8000 mg/g of dose for 3 months. It was found that peak serum curcumin concentration was achieved 1–2 h after oral curcumin intake and that its levels declined gradually within a period of 12 h. The highest (8 g/day) dose led to a peak serum concentration. Doses 8 g/day were intolerable by the patients because of the bulky volume of curcumin consumed. Curcumin administered at doses from 3600 to 8000 mg did not lead to visible toxicities except mild nausea and diarrhea. Pharmacokinetic studies of curcumin indicated a low bioavailability following oral application (Lao et al. 2006). Similarly in another study (Vareed et al. 2008), curcumin concentration declined rapidly and was unquantifiable within 3–6 h after oral intake.

In another dose-escalation study performed in the United States of America, 24 healthy volunteers were given a single dose of curcumin ranging from 50 to 200 mg (1.36–32.6 mmol) of micronized oral curcumin, along with orange juice. Their results showed absence of curcumin in the serum and only about 7 of 24 (30%) experienced side effects such as diarrhea, headache, rashes, and yellow-colored stools, which were not dose related. From this study they concluded that curcumin could be introduced safely to patients at a single dose of 12,000 mg (32.6 mmol) and at dosages of up to 8000 mg/day (21.7 mmol/day) for nearly 3 months (Strimpakos and Sharma 2008).

Baum et al. (2008) conducted a randomized, double-blind, placebo study in a group of 34 patients having Alzheimer's disease. The study participants were randomly assigned to receive curcumin at two different doses (1 or 4 g) or placebo (4 g).

Patients were randomized into 4 (10.9 mmol), 1 (2.7 mmol) (plus 3 g placebo), or 0 g curcumin (plus 4 g placebo) group. The Mini-Mental State Examination (MMSE) score that is used to assess mental status did not improve after curcumin was used for treatment. Similarly, the level of serum Aβ40 was not affected by given curcumin treatment. But curcumin administration was associated with enhanced levels of vitamin E, and curcumin also did not lead to any adverse effects during or after the treatment. Authors thus concluded that the antioxidant activity of curcuminoids might decrease the need for antioxidant vitamin E. These observations support the opening of a clinical trial of curcumin against Alzheimer's disease employing large group of patients (Baum et al. 2008).

All these studies performed and still in clinical trials provide a sound foundation for evaluating the efficacy and benefits of curcumin. However these experiments are not enough to justify the curcumin activity in AD pathogenesis. Still much is warranted to prove the efficacy of curcumin in larger human population suffering from AD.

14.9 Why Nanotechnology?

Nano-based approaches are already providing new insights to address the pathogenesis of AD. It addresses the multifaceted nature of age-related degeneration. Nanoparticles have the ability to address each phase of the disease in a highly sophisticated manner and nanotechnology has emerged as an upcoming approach for drug delivery. Further, it has opened up new landscapes in medicine, through the introduction of smart drug delivery systems, and increasing numbers of nanotherapeutics are finding a commercial pathway to reach clinical stage (Rebelo et al. 2017). Nanoparticle technology improves bioavailability, which is affected by the final particle size and stability. The bioacceptable and biodegradable nature of nanoparticles further makes them less toxic. Small size of particles helps to prolong their circulation time in blood, avoids burst release of contents, and enhances feasibility of scale-up for large-scale manufacturing (Kaur et al. 2008).

Nano-based therapeutics post oral administration are anticipated to pass through oral-cum-gastric pH barriers, promote effective absorption, and provide sustained circulation in bloodstream accompanied with successful transport across the blood-brain barrier (BBB). Based on the nature of the carrier material, nano-delivery systems can be organic (liposomes, polymers, emulsions, solid lipid NPs, dendrimers) and inorganic based like silica and carbon (Karthivashan et al. 2018). Current advancement in nanotechnology-based products presents us with opportunities to overcome the limitations being encountered. Easy and rapid penetration of drug particles helps in enhancing their overall bioavailability (Din et al. 2017).

They are being used to target drugs so as to reduce the AD symptoms or to reverse the course of the disease. Multivalency of nanoparticles further allows their functionalization with several different kinds of groups, so as to cross the BBB and

to target the site of treatment. With this particular approach enhanced drug bioavailability has been achieved in the CNS.

Nano-systems could efficiently carry and deliver drugs and other neuroprotective molecules to the brain. Physicochemical characteristics of drug moieties, such as its hydrophilicity or lipophilicity, ionization constant, molecular weight, bioavailability, extent of metabolization, and adverse effects, can result in its failure as a potential pharmacotherapeutic molecule.

Due to their small size, nanoparticles have the advantage of reaching sites which are otherwise less accessible in the body by avoiding phagocytosis and entering into tiny capillaries. Drug delivery carriers should be able to deliver the drug in a slow and sustained manner at the site of action, maintaining the therapeutic concentrations for longer time, thus reducing the frequency of administration (Das et al. 2005). Other selective transport systems include the use of ultrasound, magnetic targeting, macrophage-loaded drug formulations, carbon nanotubes, and polymeric coatings. These formulations have resulted in the efficient delivery of molecules and proteins to the relevant tissue in the central nervous system (Yallapu et al. 2015). Nano-platforms also result in enhancement of their pharmacokinetics and pharmacodynamics and exhibit minimal toxicity. Though the registry of patents for nanotechnology-based products is currently on a rise (Gulati et al. 2013; Pathan et al. 2019), clinical trials are needed to evaluate their clinical efficacy and potential toxicological effects on human health (Fan and Alexeeff 2010). In the future, neurologists and patients will benefit suitably from nanotechnology-based systems that would lead to improved therapeutic outcomes along with reduced costs.

Although currently there are no clinical studies on the use of nanotechnology to treat and cure AD, nanotechnology is predicted to alter healthcare in neurology, providing novel methods for identifying AD and thus helping in customizing a patient's therapeutic profile and thus achieving the intended goal of providing relief to AD patients.

14.10 Curcumin and Its Nanoformulations in Research and Market

Research reported in "Food Additives & Nutricosmetics" on topic "Curcumin Market Size, Share & Trends Analysis Report By Application (Pharmaceutical, Food, Cosmetics), By Region (Asia Pacific, North America, CSA, Europe, MEA), And Segment Forecasts 2018 – 2025" published in 2018 stated that "Global curcumin market size was estimated at USD 44,246.3 thousand in 2016 and is projected to register a CAGR of 13.3% over the coming time." Rising demand is projected to be driven mainly by its increasing use in food and pharmaceutical industries (Da Costa 2017). It exhibits potent anti-inflammatory and anti-oxidative properties and has emerged as a natural prophylactic option for patients suffering from late or in early stages of Alzheimer's disease. Increasing awareness among

consumers, especially in developed countries such as the USA, Denmark, and Germany, is further fuelling the demand for curcumin (Hunter 2018). There has been a heavy surge in its demand due to shifting consumer preferences toward use of more healthy and natural food products. The market potential of medical supplements is enormous on account of its availability over the counter without being restricted by any regulations. Curcumin market is expected to witness a huge development due to growth in consumer awareness regarding its effective therapeutic properties.

Additional factors such as healthy lifestyle, rising demand for organic-based food products as well as ayurvedic medicinal formulations, and innovations in pharmaceutical products have supplemented the growth of the global curcumin market. However, the formulation challenges associated with curcumin-based products are one reason affecting the growth of its market. Inclusion of curcumin and its derivatives in herbal nutritional supplements and healthcare-based products is expected to increase its popularity and demand in the coming years. Pharmaceuticals were the largest segment that accounted for more than half the share of the industry revenue in 2017. Pharmaceutical industries are dynamically trying to formulate curcumin-based medicines in the form of powder, capsules, and syrups for treatment of disorders and various health-related problems such as AD (Lee et al. 2013).

Key players functioning effectively and influencing this market currently are Biomax Life Sciences Ltd., Synthetic Industries Ltd., BioThrive Sciences, Konark Herbals & Health Care, Arjuna Natural Extracts Ltd., SV Agrofood, Star Hi Herbs Pvt. Ltd., NOW Foods, and Phyo life Sciences (https://www.bigmarket-research.com).

North America was the largest regional market in 2016, with a value of USD 23,552.9 thousand (https://www.grandviewresearch.com). Growing demand for processed food products and curcumin-based health supplements is expected to drive demand in this region. India is one of the largest manufacturers and supplier of curcumin and its products, contributing to more than 80.0% of the global production, which is on account of the presence of large-scale turmeric cultivation in India. Low consumer awareness of curcumin as a healthy ingredient in India results in majority of it being exported to North America (Hunter 2018; Skiba et al. 2018). Adroit Market Research in May, 2019, published a report on "Web Hosting Services Market will grow at CAGR of 13.25% to hit $216.59 Billion by 2025 - Global Analysis by Trends, Size, Share, Challenges, Influence Factor and Business Opportunities" and specified that Europe is expected to be the fastest growing region, with the market estimated to rise at a revenue-based CAGR of 14.8% over the forecast period. Growing demand from consumers and buyers along with the presence of strong regulatory support from the European Food Safety Association (EFSA) has made curcumin a preferred and essential pharmaceutical ingredient. A list of curcumin formulations as reported in literature which are purported to be commercially available include: (1) C95, curcumin, demethoxycurcumin, and bis-demethoxycurcumin which is 95% curcuminoid powder with uncontrolled particle size; (2) lipid curcumin particles which are composed of 20–30% of total curcuminoids, phospholipids (soy derived), vegetable stearic acid, and ascorbyl (vitamin C)

and their marketing claim that shows 65-fold better absorption than free curcumin (Gota et al. 2010); (3) hydrophilic carrier dispersed curcuminoids composed of curcuminoids (20 wt%) and antioxidants (tocopherol and ascorbyl palmitate) dispersed in water-soluble carriers such as polyvinyl pyrrolidone showing 45.9 times higher bioavailability than C95 (Deshpande and Kulkarni 2016); and (4) curcuminoid cyclodextrin complex composed of 14% curcuminoids, formulated with ~2:1 γ-cyclodextrin/curcuminoid molar ratio, and claimed to have 45-fold greater bioavailability than C95 (unpublished data) (Desai 2010; Singh et al. 2010). The obtained dispersed nanoparticulate, Theracurmin consisted of 10 w/w% of curcumin, 2% of other curcuminoids such as demethoxycurcumin and isdemethoxycurcumin, 4% of gum ghatti, and 84% of water. They shown 27 fold higher bioavailability than that of curcumin for food additive.

Past 10 years have witnessed strong and encouraging progress in the use of nanoscale-based drug delivery systems of curcumin such as loading curcumin into liposomes or nanoparticles, formulating self-microemulsifying drug delivery systems (SMEDDS), and cyclodextrin inclusions (Wang et al. 2011; Kakkar et al. 2018). Nano-based drug delivery systems provide long circulation times and improve drug's aqueous solubility and stability and their ability to cross the physiological barriers, thus improving bioavailability (Bhatia 2016). Recent research has shown the ability of nanocarriers to transport curcumin to the brain in several AD models; however, their safety, efficacy, and suitability still remain a major concern. Polymeric and lipidic nanoparticles, liposomes, micelles, and lipidic complexes are few of the nanointerventions which have been explored extensively for couturing curcumin for improved brain delivery.

A very recent study revealed the neuroprotective effect of solid lipid nanoparticles and nanostructured lipid carriers loaded with curcumin. They showed using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging that preparation processes do not have any significant effect on the antioxidant activity of curcumin. The results of this study showed promising effect for the use of curcumin-loaded NLCs in the treatment of CNS diseases. (Sadegh et al. 2019).

A very similar study conducted by us on solid lipid nanoparticles of curcumin (C-SLNs) showed an improvement in biochemical, behavioral, and histochemical changes in AD-induced Lacca mice ($AlCl_3$-induced AD). Adverse effects of $AlCl_3$ were entirely reversed by the oral administration of C-SLNs. Treatment carried out with free curcumin presented ≤15% recovery in membrane lipids (LPO) and 22% recovery in acetylcholinesterase (AChE) with respect to that of $AlCl_3$-treated group. C-SLNs group exhibited significantly improved results (97.46% and 73% recovery in LPO and AChE) at an administered dose of 50 mg/kg, and the results were comparable to those that were achieved with rivastigmine. Histopathology of the brain sections of C-SLNs-treated groups also showed significant improvement. The above study highlights the potential of C-SLNs for treatment and cure of AD (Kakkar et al. 2011).

Tiwari et al. (2013) reported that curcumin-encapsulated PLGA nanoparticles (Cur-PLGA-NPs) potentially induced neural stem cell (NSC) proliferation and differentiation in vitro and in the hippocampus and subventricular zone of adult rats, as

compared to that of uncoated bulk curcumin. Cur-PLGA-NPs induced neurogenesis by internalization of the particles into the hippocampus and significantly increased the expression of genes involved in cell proliferation and neuronal cell differentiation. Further, the authors reported an improved nuclear translocation of β-catenin and increased promoter activity of the TCF/LEF. Their results suggested that curcumin-loaded nanoparticles aided to induce adult neurogenesis through activation of the canonical Wnt/β-catenin pathway and offer a therapeutic approach (Tiwari et al. 2013).

Another work reported by Mathew et al. (2012) showed that water-soluble PLGA-coated curcumin nanoparticles were noncytotoxic and were able to destroy amyloid aggregates and exhibit enhanced anti-oxidative property. The encapsulation of the curcumin into PLGA particles also did not destroy its inherent properties and could be used for treating Alzheimer's disease (Mathew et al. 2012).

Diverse evidences have suggested the role of oxidative stress in the pathophysiology of AD; thus, Djiokeng et al. (2015) evaluated the antioxidant and anti-inflammatory activities of curcumin-loaded PLGA nanoparticles (Cur-PLGA-NP) in SK-N-SH cells (human neuroblastoma-derived cells). Cur-PLGA-NP exhibited 1.5-fold and 2.2-fold increased antioxidant activity against peroxyl radical rather than curcumin and blank nanoparticles, respectively. They also reported that Cur-PLGA-NP prevented tau phosphorylation and Akt activity, which have been shown to be altered substantially in AD brains (Djiokeng et al. 2015).

Another study by Meng et al. (2015) illustrated that low-density lipoprotein-mimic nanocarrier attached to lactoferrin encapsulated in curcumin (Lf-Mnlc-Cur). Rat model of AD was generated by an intraperitoneal injection of D-gal (0.3 mL/100 g/day for 6 weeks) and a bilateral injection of Aβ1–42 in the dorsal hippocampus (1 mg/mL; 5 μL). Fluorescent images showed that Lf-mNLC-Cur had the ability to cross the BBB and release curcumin. Hematoxylin-eosin staining also revealed a lower degree of damage in the treatment with Lf-mNLC-Cur, and a reduction in MDA content with respect to control, which suggested that the formulation was effective in decreasing the oxidative stress associated with the progression of AD (Meng et al. 2015).

Barbara et al. (2017) designed curcumin-encapsulated PLGA nanoparticle-linked peptide g7 (Cur-NP-g7) to enhance its ability to cross BBB. The authors showed that Cur-NP-g7 concentrations of 200 μM were found to be nontoxic and could internalize hippocampal neurons. Furthermore, the effect of Cur-NP-g7 on Aβ aggregation in the primary hippocampal culture derived from the rat brain, in vitro AD model showed a decrease of Aβ, thus presenting a promising tool for the treatment of AD (Barbara et al. 2017).

In a study undertaken by Huang et al. (2017) PLGA nanoparticles conjugated with the S1 peptide (an inhibitor of Aβ generation), brain-targeting calreticulin (CRT) (a peptide that binds to the transferrin receptor), and curcumin revealed that the nanocarrier (S1-CRT-NP+Cur) was taken up into a cellular model of the BBB (brain microvascular cells), suggesting that it could permeate across the BBB. Their results of Y-maze and new object recognition test demonstrated that PLGA nanoparticles significantly improved the spatial memory and recognition in transgenic AD

mice. Further they remarkably decreased the level of Aβ, reactive oxygen species (ROS), TNF-α, and IL-6 and enhanced the activities of super oxide dismutase (SOD) and synapse numbers in the AD mouse brains. Compared with other PLGA nanoparticles, CRT peptide-modified PLGA nanoparticles co-delivering S1 and curcumin exhibited most beneficial effect on the treatment of AD mice, suggesting that conjugated CRT peptide and encapsulated S1 and curcumin exerted their corresponding functions for the treatment (Huang et al. 2017).

In a recent report by Fan et al. (2017), PLGA-PEG nanoparticles encapsulating curcumin and attached to the B6 peptide (PLGA/PEG-B6-Cur) were designed. PLGA-PEG was employed to enhance the bioavailability, and the B6 peptide was employed so as to allow BBB crossing. In order to explore and test their potential for usefulness in AD, PLGA/PEG-B6-Cur were proven in HT22 cells and an AD rodent model (APP/PS1 transgenic mice). Cytotoxicity studies in HT22 cells demonstrated that PLGA/PEG-B6-Cur did not affect cell viability up to a concentration of 500 μg/mL. The results of experimental studies revealed a significant improvement in cognitive performance, which correlated well with a decrease in tau phosphorylation and Aβ production in the hippocampus (Fan et al. 2017).

This data is a proof of the potential of curcumin and its nanocoutured formulations to forestall or manage neurodegenerative disorders especially AD.

14.11 Conclusions

Huge database, patents, and preclinical and clinical studies on curcumin have established the potential of this molecule in several diseases including Alzheimer's disease. Its therapeutic potential has been further evidenced by making into nano-mediated drug delivery systems. These nano-therapeutics which include the polymeric/lipidic/conjugated nanoparticles, liposomes, and micelles have been able to reach the market in nutraceutical capacity and not as drugs. However one can foresee its transition as a drug once the in vitro-in vivo correlations are achieved in clinical phase of the curcumin trials. Future studies in this area are required to elevate the status of nano-curcumin as therapeutic in AD.

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Chapter 15
Nanopharmaceuticals for the Improved Treatment of Cerebral Stroke

Shagufta Khan, Aarti Belgamwar, and Pramod Yeole

Abstract Stroke remains the leading cause of death and disability across the globe. However, there is a dearth of effective therapy for its treatment. Over the past decade, use of nanomedicine has gained overwhelming interest for the treatment of cerebral stroke due to the constant failure of the conventional treatment. The most widely investigated nanocarriers include neuroprotective agents loaded on functionalized liposomes and polymeric nanoparticles for targeted delivery to the brain, metal oxide nanoparticles, carbon nanotubes, dendrimers, and scaffolds. This chapter will focus on the investigations undertaken hitherto on different types of nanocarriers for delivery of therapeutic agents for the treatment of stroke.

Keywords Nanopharmaceuticals · Cerebral stroke · Nanomedicine · Liposomes · Nanocarriers · Neuroprotective

Nomenclature

BBB	Blood–brain barrier
bFGF	Basic fibroblast growth factor
GBD	Global Burden of Disease
hEPC	Human endothelial progenitor cells
MSCs	Mesenchymal stem cells
PEG	Polyethylene glycol
PLA	Poly(lactic acid)
PLGA	Poly(lactic-co-glycolic acid)
ROS	Reactive oxygen species
SLN	Solid lipid nanoparticles

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TfR	Transferrin receptor
tPA	Tissue plasminogen activator
uPA	Urokinase-type plasminogen activator
VEGF	Vascular endothelial growth factor

15.1 Introduction

Stroke is one of the leading causes for disability among elderly subjects and third largest cause of morbidity in developed nations. Stroke and dementia related cases are progressively increasing because of increasing aging population.

The Global Burden of Disease (GBD) project has shown a decline in infectious and nutritional disorders, and a rise in non-communicable diseases, like stroke, heart disease, cancer, diabetes mellitus, and chronic obstructive pulmonary disease. The GBD project estimates that during the last two and half decades the number of people with incident of stroke has increased to 100%. Projections demonstrate that the non-communicable diseases will be increasingly prevalent in the next decades and may reach epidemic proportions, which will seriously influence global public health (Feigin et al. 2017). These estimates clearly indicate that currently used primary stroke prevention strategies are not sufficiently effective and require a serious revision.

Stroke occurs when blood flow to the brain is blocked, resulting in cell death. There are two main types of stroke: hemorrhagic stroke, which results from blood vessel rupture, and ischemic stroke, which results from blood vessel occlusion. Fifteen percent of the total stroke cases are due to hemorrhage while more than 80% are due to ischemia.

Brain depends on oxidative phosphorylation to provide energy, and thus requires high amount of oxygen and glucose. Impairment of cerebral blood flow cuts off the delivery of key nutrients and oxygen to the brain, compromising the energy consumption needed to maintain homeostasis. Reduced blood flow (Fig. 15.1) initially causes loss of electrical activity in neurons, with other cellular functions mostly unaffected (Astrup et al. 1977). If ischemia persists beyond approximately 30 min (Lipton 1999), vital cell functions, such as membrane ion pumps, start failing, leading to morphological changes and, eventually, cell death (Bell et al. 1985). This series of process yields a core of dead tissue, temporarily surrounded by the penumbra (Fig. 15.2), consisting of cells that have lost their electrical activity but not their other functions. Cells within the penumbra are quite retrievable with return of blood flow.

This chapter aims to highlight the limitations of the current strategies for treating stroke and progress in nanomedicine based strategies. It will also focus on the challenges in translating these strategies from lab-to-clinic.

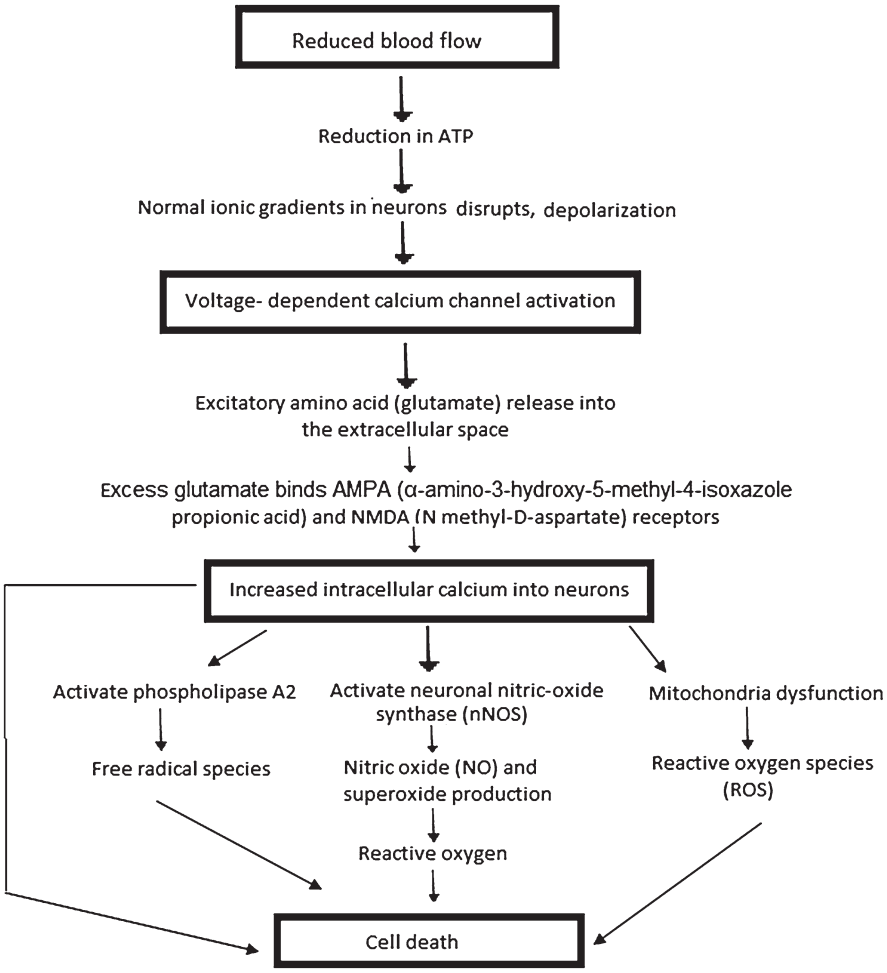

Fig. 15.1 Events during ischemic cerebral stroke

15.2 Current Ischemic Stroke Treatment

The ischemic stroke intervention involves an early dissolution of intravascular blood clots and cleavage of the fibrin network within the thrombus, so that the downstream damage and ischemia of the brain tissue could be avoided (Torno et al. 2008). Thrombolytic (recanalization) and neuroprotective therapy are the two main strategies for ischemic stroke intervention (Zheng et al. 2015).

Urokinase-type plasminogen activator (uPA) or recombinant tissue plasminogen activator (rt-PA) is the thrombolytics approved by FDA (Adibhatla and Hatcher 2008). Clinical trials have demonstrated that patients treated by thrombolytic agents within the first 3 h of onset of acute ischemic stroke are able to achieve functional

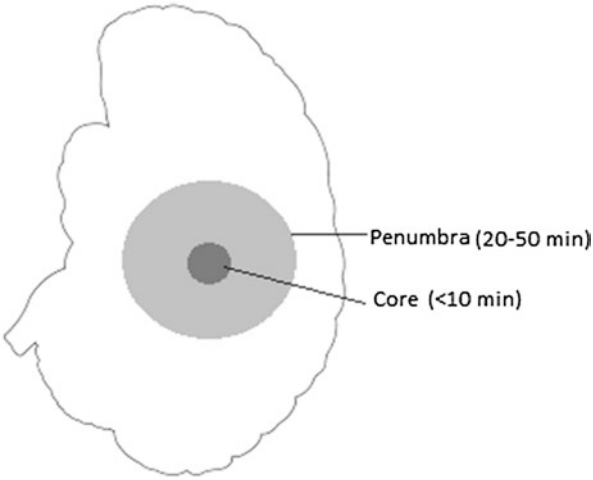

Fig. 15.2 Core of dead tissue surrounded by penumbra

independence in the weeks or months (Kamat et al. 1996). However, symptomatic intracerebral hemorrhage occurs in about 6.4% patients after an intravenous injection of rt-PA due to the low specificity of thrombolytics. Also, the therapeutic time window of rt-PA is short (within 4.5 h after stroke symptoms) (Hacke et al. 2008). Therefore, there is a need for the targeted delivery of thrombolytic agents, which have the potential to increase their specificity, reduce side effects, and enhance circulation time of the thrombolytics.

The neuroprotective therapy involves blocking of proinflammatory cytokines and cell adhesion agents, decreasing of lipid peroxidation processes, and blocking of apoptosis. More than 1000 molecules broadly classified as neuroprotective, aiming to stop or slow the secondary damage associated with the ischemic cascade following stroke, have shown promise in the initial stages of research but have failed to demonstrate efficacy in clinical studies because of inadequate efficacy or side effects. The clinical failure of most neuroprotectors studied may be because of (1) their poor penetration through the blood–brain barrier (BBB) into the region of cerebral ischemia, (2) significant decrease in the efficiency of neuroprotectors due to concomitant diseases like diabetes mellitus, arterial hypertension, vascular dementia, and aging dysfunction of brain metabolism, etc., (3) the heterogeneity in location and intensity of cerebral ischemia requires the use of different drugs or different doses of a drug for reperfusion processes, and (4) difficulty to analyze and standardize patients in groups and to optimize the effect of a drug. Thus, there is an urgent need of an alternative treatment strategy that has the capability to restore the blood flow and reduce the secondary damage to penumbra.

15.3 Nanomedicine in Ischemic Stroke Treatment

In recent years, there has been considerable interest in the application of nanomedicine for the treatment of stroke (Kubinova and Sykova 2010). The important reasons for their popularity are (1) capability of the nanomedicine to decrease neurotoxicity by decreasing the dose; (2) ability to cross the blood–brain barrier and ability to accumulate in the ischemic region (Ishii et al. 2013); (3) target the clot by means of ligand based targeting; (4) release the drug from the carrier under specific stimuli (Fukuta et al. 2015). Liposomes have been at the top of these investigations followed by polymeric nanoparticles owing to their safety, biodegradability, and biocompatibility.

15.3.1 Transport of NPs through the BBB

The major limiting factor in the treatment of stroke is the poor diffusion of drugs through the BBB. Nanotechnology has shown success in achieving high levels of drug in the brain with the help of nanocarriers. The pathways by which the nanocarriers can cross the BBB are (1) by paracellular transport; during stroke, there is disruption of the BBB disruption because of which even bigger NPs (few hundred nm) can pass through the barrier paracellularly, (2) binding with the carriers like glucose transporters, e.g., transport of mannose coated liposomes using GLUT1, (3) by receptor-mediated endocytosis where surface of the NPs is functionalized with ligands which can bind on receptors expressed at the BBB like transferring, insulin like growth factor, and leptin receptors, e.g., PEGylated liposomes coupled with transferring, and (4) by adsorptive transcytosis of charged nanoparticles, e.g., charged polystyrene NPs (Fig. 15.3).

15.3.2 Liposomes for Ischemic Stroke

The immense progress in liposome technology has led to the advent of long circulating liposomes and functionalized liposomes for active targeting. Long circulating liposomes are most widely used in the management of ischemic stroke. Prolonged circulation had been achieved by reducing the size ($^{<}$100 nm) or by surface modification with PEG. PEGylated liposomes were shown to accumulate in the ischemic brain hemisphere at an early stage after ischemia reperfusion.

Tissue plasminogen activator (tPA), a proteolytic enzyme which enhances the conversion of plasminogen to plasmin, degrades the fibrin matrix in the clot and improves blood flow to the ischemic region, was loaded on liposomes to circumvent its short half-life (2–6 min) and improve specificity (therapeutic time window of tPA is less than 4.5 h after this if administered, might lead to significant hemor-

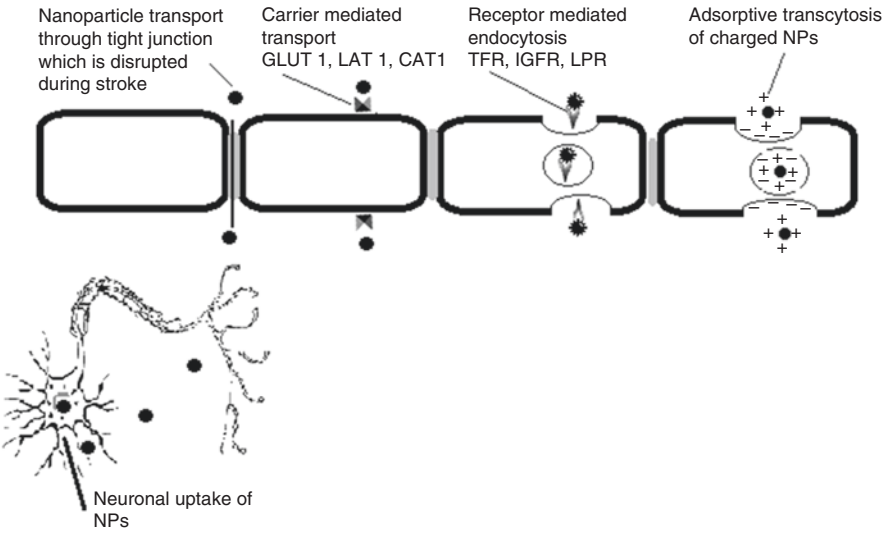

Fig. 15.3 Pathways of NPs transport through the BBB. *GLUT* glucose transporter, *LAT* large neutral amino acid transporter, *CAT* cationic amino acid transporter, *TFR* transferring receptor, *IGFR* insulin like growth receptor, *LPR* leptin receptor

rhagic complications). Hemorrhage was reduced when tPA loaded in actin-targeted liposomes was intravenously administered by the internal carotid artery in an in vivo model bearing clots (Asahi et al. 2003).

However, endovascular recanalization and intravenous administration of tPA does not address cellular damage to brain tissue adjacent to the infracted area, known as the stroke penumbra.

Passively targeting the BBB on the principle of EPR effect by loading these neuroprotective molecules on liposomes may improve results (Table 15.1). Intravenous administration of PEGylated liposomes loaded with tacrolimus, a neuroprotective agent which is also a P-gp substrate (Loscher and Potschka 2005), before secondary cerebral damage known as ischemic/reperfusion (I/R) injury resulted in a significantly suppressed cerebral cell death. While, the damage volume for PEG-liposomes encapsulating tacrolimus was about 0.2 cm^3; for free tacrolimus and PBS, it was ~0.3 and ~0.4 cm^3, respectively. This formulation also suppressed superoxidative anions induced-damage in the brain and improved motor function deficits compared to free tacrolimus.

Fasudil, a Rho-kinase inhibitor is approved for cerebral vasospasm after subarachnoid hemorrhage. Because of its neuroprotective properties, it was tested for the treatment of ischemic stroke. However, phase III clinical trials showed poor clinical efficacy, short retention in the bloodstream, and difficulty to penetrate the BBB of fasudil (Fukuta et al. 2016). Therefore, it was encapsulated in PEG-liposomes and intravenously administered in middle cerebral artery occlusion (MCAO) rats (Fukuta et al. 2016). Fasudil-loaded PEG-liposomes diffused and

Table 15.1 Liposomes and carbon nanotubes investigated in stroke

Neuroprotective agent tested	Type of liposome/ carbon nanotube tested	Findings	Reference
t-PA/ dexamethasone	PEGylated liposomes	Intravenous injection in middle carotid artery occlusion rat model, showed improvement in behavioral outcome	Tiebosch et al. (2012)
Citicoline	Targeted PEGylated immunoliposomes labeled with gadolinium	Intravenous injection in the animal model with permanent intracranial occlusion of middle cerebral artery, revealed accumulation of 80% vectorized liposomes in the periphery of the ischemic lesion as detected by MRI and reduction of lesion volumes up to 30% in comparison to animals treated with the free drug	Agulla et al. (2014)
Simvastatin	PEGylated liposomes	Intravenous injection of neutral and negatively charged liposomes were found to accumulate and deliver simvastatin in the infracted area of transitory middle cerebral artery occlusion animal model	Campos-Martorell et al. (2016)
Cyclosporine	PEGylated liposomes	Intravenous injection of CsA-liposomes showed recovery of the infarct size, brain edema and neurological activities, and inhibition of inflammation in transitory middle cerebral artery occlusion animal model	Partoazar et al. (2017)
Hemoglobin	PEGylated liposomes	Intravenous administration of liposomal hemoglobin to middle cerebral artery occlusion animal model reduced the area of histological damage in the brain cortex	Kawaguchi et al. (2017)
Paired immunoglobulin-like receptor B ectodomain (sPirB)	PEGylated liposomes labeled with NIR probe	Intravenous administration of sPirB-containing liposomes in transitory middle cerebral artery occlusion animal model revealed accumulation in the ischemic region and improved recovery, showing potential for a new theranostic platform	Wang and Tang (2018)
Multi walled-carbon nanotubes (MWCNT)	Hydrophobic MWCNT impregnated with subventricular zone neural progenitor cells (SVZ NPCs)	HP CNT-SVZ NPC transplants (microinjection into striatum post ischemia) improved rat behavior and reduced infarct cyst volume and infarct cyst area	Moon et al. (2012)
Fullerene	Polyhydroxylated fullerene	Administration of fullerene nanoparticles in transitory middle cerebral artery occlusion animal model, significantly decreased the infarct volume and inhibited brain oxidative/nitrosative damage	Vani et al. (2016)

accumulated in the I/R region, from an early phase after administration up to 24 h. Moreover, it significantly suppresses the volume of damaged brain tissue, obtaining ~0.2 cm^3, compared to free fasudil (~0.3 cm^3) and PBS (~0.4 cm^3). Fasudil-loaded PEGylated liposomes also reduced in a significant manner neutrophil invasion and improved the motor functional disorder. The success of this formulation was basically due to PEGylation and liposome size. Using the same stroke in vivo model, it was confirmed that ~100 nm PEG-liposomes got a higher accumulation on the ischemic side, ~200 nm PEG-liposomes showed a lower accumulation, and no accumulation was observed for ~800 nm PEG-liposomes. These results were attributed to the disruption of the BBB and the leakage of liposomes to the brain parenchyma, where they gradually accumulated in the ischemic region via the EPR effect.

Xenon is a pleiotypic cytoprotective gas, which rapidly diffuses across the BBB. However, its administration by inhalation requires intubation and ventilation with a large xenon concentration that reduces the maximum fraction of inspired oxygen (Peng et al. 2013). Thus, in a study it was encapsulated into echogenic liposomes and systemically administered in t-MCAO rats. It was demonstrated that this formulation effectively reduced ischemic neuronal cell death and improved neurological function.

Specificity of PEGylated liposomes can be further enhanced by conjugating receptor specific ligand on the surface of liposomes so that active targeting to the ischemic region is possible. Vascular endothelial growth factor (VEGF) was loaded in PEGylated liposomes with surface linked to transferrin and intravenously administered 2 days after inducing a t-MCAO model (Zhao et al. 2011). VEGF confers neuroprotection, promotes neurogenesis and cerebral angiogenesis, and transferrin is an iron-binding glycoprotein with high affinity for the transferrin receptor (TfR) at the BBB (Hatakeyama et al. 2004). While the damage volume for VEGF-loaded PEGylated liposomes coupled to transferrin was about ~2.5 cm^3, for VEGF-loaded PEGylated liposomes was ~3.0, and for saline ~3.5 cm^3, respectively. VEGF-induced neovascularization in the penumbra zone was significantly higher for the actively targeted formulation (245,873 microvessels per field) than for the passively targeted formulation (139,801.3) and for saline (102,175.5).

Despite the improved pharmacokinetic and bio-distribution results with liposomes, there is no successful liposomal formulation yet in the clinic. The lack of translation of liposomal formulation from bench-bed necessitates design of more optimized formulation for ischemic stroke management.

15.3.3 Polymeric Nanoparticles

Studies suggest that deficiency in energy during cerebral ischemia causes a cascade of events like endothelial swelling, lactic acidosis, increased permeability caused by decreased occludin and zonula occludens-1 expression, induction of proteases which degrade the extracellular matrix, formation of reactive oxide species, and leukocyte extravasation that can result in BBB disruption. Because of the BBB dis-

ruption, moderately bigger nanoparticles (a few hundred nanometers) are said to pass through the BBB (Nance et al. 2012). In a study, Ferreira et al. (2016) investigated retinoic acid loaded polymeric NPs on vascular modulation and endothelial cell differentiation after ischemic stroke. Retinoic acid is a strong regulator of neurogenesis by actively promoting neural stem cell differentiation, and of angiogenesis by stimulating wall formation via VEGF enhanced signaling. However, retinoic acid has low aqueous solubility, fast degradation, and narrow therapeutic window; therefore, its therapeutic efficiency is low. Thus to achieve the full therapeutic potential, retinoic acid loaded polymeric nanoparticles using dextran sulfate and polyethyleneimine were developed. Retinoic acid loaded NPs were tested against human endothelial progenitor cells (hEPC) isolated from the peripheral blood of large vessel occlusion and non-lacunar ischemic patients and were found to be about 83-fold more efficient than free retinoic acid in enhancing hEPC proliferation. The brain-targeted chitosan nanoparticles were loaded with the basic fibroblast growth factor (bFGF) and their surface was functionalized by conjugating with a small peptide inhibitor of caspase-3 (z-DEVD-FMK) directed against the transferrin receptor-1 on the brain endothelia to induce receptor-mediated transcytosis across the BBB. The nanoparticles significantly decreased the infarct volume after 2-h middle cerebral artery occlusion and 22-h reperfusion in mice (Jin et al. 2013). Curcumin loaded solid lipid nanoparticles (SLN) were reported to have an ability to penetrate the BBB and improve the bioavailability of curcumin. The SLN were found effective against cerebral ischemic injury (Kakkar et al. 2013).

15.3.4 Metal and Metal Oxide Nanoparticles

Studies have shown that some metallic NPs can function as scavengers of reactive oxygen species (ROS) (Takamiya et al. 2012). Platinum NPs have been reported to have free radical scavenging effect, it was found to exhibit antioxidant effect by scavenging superoxide anions as well as hydrogen peroxide, when tested in vivo (Takamiya et al. 2012). Due to the large surface area to volume ratio, platinum NPs have high electron density at the surface which helps in quenching both superoxide anions and hydrogen peroxide. The ROS scavenging property is also presented by cerium oxide NPs which have been used as metal coatings to reduce oxidation. Cerium oxide NPs represent a potential new treatment for stroke. These cerium oxide NPs provided a significant neuroprotective effect on adult rat's spinal cord neurons. A study by Estevez et al. (2011) showed that cerium oxide NPs can reduce ischemic cell death on a mouse hippocampal brain slice model of cerebral ischemia (Fig. 15.4). The ability of cerium NPs to bind reversibly with oxygen and shift between Ce^{4+} and Ce^{3+} under oxidizing and reducing conditions plays important role in scavenging reactive oxygen species. The oxidized Ce^{4+} at the surface dismutates the superoxide radical to form hydrogen peroxide which is consequently disproportionated to molecular oxygen and water. In addition, both cerium and yttrium oxide NPs have antioxidant properties and they are found to reduce pools of

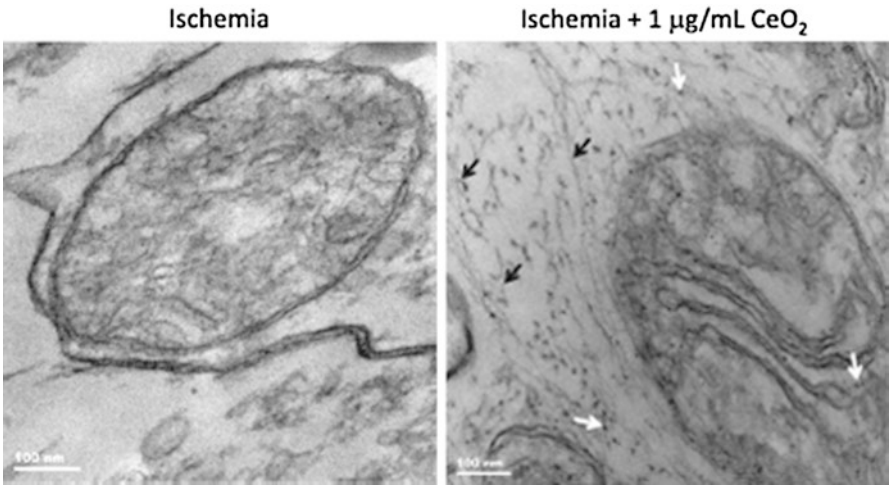

Fig. 15.4 TEM micrographs demonstrating the location of cerium oxide nanoparticles within hippocampal brain slices. The hippocampal brain slices shown were exposed to 30 min of ischemia and allowed to recover for 24 h. Nanoceria (white arrows) are located in high densities in the mitochondria and associated with neurofilaments (black arrows). Note the relative disorganization of the mitochondrial cristae in the ischemic slice compared to the more normal-appearing mitochondrial cristae in the slice treated with identical ischemia but in the presence of nanoceria (copyright requested)

preformed ROS in HT22 cells (Schubert et al. 2006). Thus, metal or metal oxide NPs can be potentially used as a novel therapeutic agent against oxidative injury to the ischemia brain.

15.3.5 Nanofiber Scaffolds and Self-Assembling Peptide Nanofibers

Nanofiber scaffolds are produced by electrospinning from either natural biomaterials like collagen, fibrin, hyaluronic acid, alginate, and fibronectin, or synthetic macromolecules such as poly(lactic acid) (PLA), polyethylene glycol (PEG), and poly(lactic-co-glycolic acid) (PLGA). Nanofiber scaffolds can serve as a permissive bridge for axonal regeneration or as cell/drug carriers. However, there are some inherent problems with the traditional nanofiber scaffolds. Peptide nanofiber scaffold prepared via self-assembling process is an area of growing interest for CNS regenerative medicine (Ellis-Behnke and Schneider 2011; Kubinova and Sykova 2010). Self-assembly is one of the most powerful ways to prepare nanostructure materials and offers great opportunities for the creation of novel biomaterials.

Self-assembling nanofibers are synthesized from peptide amphiphile molecules. These peptides have periodic repeats of alternating positive and negative l-amino

acids, which in the presence of a physiological salt-containing solution spontaneously self-assemble from the aqueous solution to form a stable nanofiber matrix (Zhang et al. 2005). These peptides can be designed to incorporate specific functional ligands, such as peptide epitopes containing integrin receptor binding sites, for example, RGD, IKVAV, or RADA, which impart active targeting capability. The advantages of self-assembling peptides over natural and/or synthetic materials are: (1) self-assembling peptide scaffold is designed from bioactive matrix without use of exogenous proteins, posing a minimized risk of immunological reaction; (2) self-assembling peptides are amenable to functionalization to mimic the naturally occurring proteins; (3) self-assembling peptides allow for high cell implantation densities and show highly potential for controlled drug release (Ellis-Behnke and Schneider 2011; Luo and Zhang 2012).

15.3.6 Superparamagnetic Iron Oxide Nanoparticles

Superparamagnetic iron oxide nanoparticles for the delivery of siRNA and tracking of endothelial progenitor cells have been studied due to their potential in ischemic stroke therapy.

Stem cell transplantation has emerged as a promising therapeutic strategy for ischemic stroke, owing to their inherent capacity of self-renewing (Trounson and McDonald 2015; Thwaites et al. 2012). The use of mesenchymal stem cells (MSCs) has many advantages, such as the simplicity of isolation and expansion, and biological properties of secreting many bioactive immunoregulatory and pro-regenerative macromolecules (Caplan and Dennis 2006), and they have been widely used in regenerative therapies (Caplan 2007). Superparamagnetic iron oxide nanoparticles (SPION) can be used as a carrier for stem cells as well as for their tracking. To improve their cellular uptake, SPION surface can be functionalized. In a study SPION was coated with hydrophyllic polysaccharide amylose. Amylose has such versatile traits like biocompatibility, biodegradability, capacity to be easily modified, and improved transfection efficiency. Nanometer-sized SPIONs-complexed amylose nanoparticles cationized with spermine were found to be highly effective and safe carrier for the transfer of stem cells (Lin et al. 2017).

15.3.7 Dendrimers

Estrogen-dendrimer conjugates are reported to provide strong neuroprotection in the hippocampal region of the brain by the activation of extracellular signal regulated kinase—protein kinase B—cAMP response element-binding—brain-derived neurotrophic factor signaling (Yang et al. 2010). Biodegradable poly amido amine dendrimers had been used as a means to accomplish siRNA mediated gene knock down with the subsequent reduction in infarct volume (Gilmore et al. 2008). Johnson

et al. (2010) have reported the neuroprotective capability of S-nitroso-N-acetylpenicillamine-derivatized generation4 polyamidoamine (G4-SNAP) dendrimers against ischemia/reperfusion (I/R) injury in an isolated, perfused rat heart. G4SNAP dendrimers have also been used for delivery of nitrosothiol for enhanced nitric oxide (NO) release and glutathione for the treatment of cerebral ischemia as there is significant depletion in glutathione following ischemic injury (Taskin et al. 2009).

15.3.8 Nanotubes

Single-walled nanotube—neuron hybrid system stimulates brain circuit activity and may enhance regenerative processes of damaged neurons through improved neural signal transfer, dendrite elongation, and cell adhesion (Lovat et al. 2005). The nanoscale dimension of nanotubes allows them to interact neurons at the molecular level and establish electrical interfacing with neural cells (Mazzatenta et al. 2007). Because of the high electrical conductivity, nanotubes can act on the amplitude and shape of the signals and increase the efficiency of neuronal signal transmission (Massobrio et al. 2008). Multi-walled carbon nanotubes modified by amino groups, when added with nerve growth factor (NGF), were found to promote outgrowth of neuronal neurites in dorsal root ganglion neurons and rat pheochromocytoma cell line in culture media (Matsumoto et al. 2010).

15.3.9 Carboxyfullerenes

Fullerenols, derivatives of hydroxyl-functionalized fullerenes, have therapeutic properties in several acute or chronic neurodegenerative diseases. They are known to function as free radical scavengers and reduce excitotoxicity and apoptosis induced by glutamate, NMDA (N-Methyl-D-aspartate), and AMPA (alphaamino-3-hydroxy-5-methyl-4-isoxazole-propionic acid) (Dugan et al. 1996). The fullerenols provide neuroprotective effects by blocking glutamate receptors and lowering the intracellular calcium. Water-soluble regioisomers of fullerenes, containing malonic acid groups, were found to be free radical scavengers and inhibitors of excitotoxic and ischemic death of cultured cortical neurons. The C3 regioisomer of C60 enters lipid membranes, and, reduces NMDA receptor mediated toxicity and apoptotic neuronal death (Dugan et al. 1997).

15.4 Nanomedicine in Intracerebral Hemorrhage Treatment

Intracerebral hemorrhage (ICH) accounts for 10–15% of all strokes, which leads to a substantial degree of mortality (30–50% within 6 months) and disability (approximately 80% within 6 months) (Mayer and Rincon 2005). The ICH treatment mainly involves delivery of neuroprotective agents. The most widely investigated nanocarriers for ICH treatment are polymeric nanoparticles and scaffolds. To achieve active targeting at the site of stroke they have been functionalized with ligands specific to the receptors expressed at the BBB.

15.4.1 Polymeric Nanoparticles

Several studies suggest that small sized nanoparticles can cross BBB (Krol et al. 2013). In a study it is reported that poly (ethylene glycol) (PEG)-coated nanoparticles of 85 nm in diameter can rapidly distribute within the brain. Further, it is proposed that a secondary injury during intracerebral hemorrhage caused by the products of coagulation and hemoglobin breakdown, involves the release of thrombin, ferrous iron, hemin, and halotransferrin which induce breakdown of the BBB. Because of the BBB disruption, moderately bigger nanoparticles (a few hundred nanometers) are said to pass through the BBB. The neuron growth factors and brain-derived neurotrophic factors (BDNF) loaded nanoparticles have been developed for ICH intervention. A non-viral gene delivery system comprising of polybutylcyanoacrylate (PBCA) nanoparticles, cytomegalovirus (cmv), Neurotrophin-3 (NT-3), and hormone response element (HRE) was used to treat ICH in rat model (Chung et al. 2013).

Two types of redox polymer nanoparticles, poly (ethylene glycol) (PEG)-b-poly[(2,2,6,6-tetramethylpiperidine-1-oxyl)aminomethylstyrene] and PEG-b-poly [(2,2,6,6-tetramethylpiperidine-1oxyl) oxy-methylstyrene], were prepared to treat the focused ultrasound-induced ICH, and the results indicated that redox nanoparticles targeting free radical pathways could decrease acute ICH-induced brain edema and neurologic deficit.

15.4.2 Scaffolds

Surgery is considered as a lifesaving measure for the patients with cerebellar hemorrhage or large hematomas (Hemphill et al. 2015). It has the potential to reduce the mass effect of hematoma and stop the secondary injuries, but rebleeding after surgery would increase hematoma volume. A self-assembling peptide (SAP) of RADA16-I nanofiber scaffold was synthesized to prevent postoperative hematoma growth (Sang et al. 2014). SAP displayed the good hemostatic and regenerative

effects in vivo, thus, the combined treatment with hematoma removal and locally delivered SAP nanofiber scaffold could prevent rebleeding after surgery, decrease hematoma volume, brain edema, perihematomal inflammatory cell infiltration, and apoptosis, as well as improve functional recovery (Mozaffarian et al. 2015).

Targeted delivery of drugs in hematoma has drawn considerable attraction for the ICH intervention. A fibrinbinding domain (FBD) was fused to BDNF for the targeted delivery. FBD-BDNF scaffold displayed a specific binding ability to fibrin and used the hematoma as the scaffold after ICH (Han et al. 2011). In the ICH animal model, FBD-BDNF was found to concentrate and retain at the hematoma, significantly reduce the hematoma volume, reduce loss of tissue, and promote neural regeneration, improving the overall rat behavioral performance.

Scaffolds are essentially suitable for local intervention. Combining with the ICH hematomas cleaning operation, scaffolds can be implanted into the hemorrhagic cavity and improve the postoperative outcome by alleviating the secondary injuries.

15.5 Conclusion and Future Perspectives

Nanomedicines represent highly promising option for treating cerebral stroke. The ability of the functionalized NPs and liposomes to cross the BBB and deliver the therapeutic agent effectively to the target site has drawn a great deal of attention. However, significant challenges still exist in translating nanomedicine from bench to bedside for treating stroke. Most of the studies are still in the preclinical stage; therefore, there is a need of multiple studies in humans to understand the distribution, biodegradability, toxicity, and targeting efficiency of nanocarriers.

In order to address these vital issues which are yet not resolved and to justify positive benefit-to-risk ratio unequivocally, acute and chronic toxicological assessment of nanocarriers on a case-by-case basis is necessary before they are approved for patient use. Despite the challenges, with the enormous pace and advancement in nanomedicine research, in future, nanotechnology will certainly evolve into the most promising drug delivery technique for cerebral stroke treatment.

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Index

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