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Beyond the blood-brain barrier: the use of nanoparticles in drug delivery

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List of Abbreviations:

APP - Amyloid precursor protein

BBB – Blood brain barrier

CNS – Central nervous system

CRT- Calreticulin

EC – Endothelial cell

FDA – U.S. Food and Drug Administration

IN - Intranasal

IV – Intravenous

MCAO - Middle cerebral artery occlusion model

NLC – Nanostructural lipid carrier

NP- Nano particle

NVU – Neurovascular unit

PAMAM – Poly-amidoamine

PLA – Poly (lactic acid)

PEG – Poly (ethylene glycol)

PLGA - Poly (lactic-co-glycolic acid)

ROS – Reactive oxygen species

SLN – Solid lipid nanoparticle

3-NP – 3-nitropropionic acid

1. Introduction

Given that the brain is the most sensitive organ it requires a tightly regulated microenvironment. The blood brain-barrier maintains brain homeostasis and protects it from unwanted or potentially dangerous molecules. This is possible due to the distinctive characteristics of the BBB such as less fenestration, tight junctions, pericytes and astrocyte foot processes [1]. However, this peculiar structure plays the role of a double edge sword as it also prevents most modern drugs from crossing into the CNS. This means that brain disease therapy is severely hampered [2].

Neurodegenerative disease incidence is projected to rise substantially in the coming decades. The explanation revolves around the fact that many of these diseases are associated with old age and the world population getting older. It is thought that by 2040 neurodegenerative diseases will represent the second most common cause of death in humans, after cardiovascular disease. Alzheimer's disease alone will affect as many as 106 million individuals by 2050 [3]. Treatment of these disorders is, to this day, difficult due to hurdles such as the BBB. Nevertheless, recently focus has been shifted towards novel methods of drug delivery in the CNS such as nanomedicines. They are an encouraging method that has the potential to surpass the efficiency of current drug delivery methods by protecting the drug they are carrying and delivering it to the brain. Furthermore, a very enticing aspect of nanomaterials is their adaptability [4]

The most studied NPs are polymeric nanoparticles such as PLGA and PLA, along with liposomes and inorganic particles like gold. PLGA and PLA have been of particular interest due to their biocompatibility, stability and ability to be conjugated with other molecules. They can also be loaded with a variety of agents besides medicines. For example, nucleic acids, diagnostic agents and certain proteins. They have even been approved by the FDA. The other extensively researched area of nanoparticles, liposomes, are also biodegradable and biocompatible; however, it is important to note their ability to carry both hydrophilic and hydrophobic drugs [5].

2. The Blood Brain Barrier

The blood brain barrier (BBB) is the name given to the unique structure enveloping the central nervous system's (CNS) vasculature. Its main role is to maintain homeostasis in the CNS for optimal function and protect it against pathogens, toxins and inflammation. This is achieved through the tight regulation of the interaction between the CNS and the blood [6]. Furthermore, the BBB regulates the essential nutrient uptake of the brain for molecules such as glucose, hormones, vitamins, insulin and leptin to ensure the appropriate metabolism of the CNS [1]. The only areas of the brain lacking the BBB include the area postrema, median eminence, pineal gland, neurohypophysis, the subfornical organ and the lamina terminalis [7].

The first observation of the blood brain barrier was noted by Paul Ehrlich in his 1885 study when he injected water soluble dyes like trypan blue in the circulatory system. He found that the dyes stain all organs besides the brain and spinal cord. Further studies conducted by Goldmann and Lewandowsky in the early 1900's consolidated the existence of a barrier between the central nervous system and the rest of the body. Nevertheless, the BBB's structure and existence were debated until the 1960's [8] when the BBB was first localized by electron microscopy [1] using horseradish peroxidase administered to mice via the IV route [9].

2.1 Anatomy of the blood brain barrier

The core of the BBB is composed of its inner most layer, the EC layer with its adherens and tight junctions and basement membrane. Other elements surrounding the basement membrane include pericytes and astroglial foot processes. The close interactions between the components of the BBB and neural cells namely, neurons and microglia, form a functional unit called the neurovascular unit. Understanding the properties of the neurovascular unit is crucial in grasping brain functions.

The EC layer is made up of single specialized cells expressing an increased number of mitochondria [10]. The luminal surface of these cells is lined with a layer of glycocalyx while the abluminal side presents an irregular pattern of pericytes [1]. A physical barrier is created between the blood and the neural parenchyma due to the complex tight

junctions found between these cells. The tight junctions contain transmembrane proteins such as occludins, claudins and junction adhesion molecules that close the interconnecting spaces between the cells [11]. This diverts most of the molecules towards a transcellular route across the BBB rather than the usual paracellular approach. Gaseous molecules like oxygen or carbon dioxide can freely pass through the BBB. Similarly, some lipophilic drugs like barbiturates can also cross the barrier [12]. Despite this, most drugs we know of today cannot penetrate the barrier which heavily hinders the progress of both brain disease treatments and imaging techniques. Due to their large size, none of the current biotechnology drugs can pass the barrier. Moreover, a further estimate of 98% of small molecules are also blocked [2].

Pericytes have been recently discovered to be key players in BBB regulation. They wrap around 40% of the abluminal surface of the neurovascular surface and are fully embedded in the basement membrane. Although physically separated from the EC, communication is still possible through peg socket, gap an adhesion junctions. The roles of pericytes include barrier genesis, vessel stabilization [11] as well as control of the vessel diameter and cerebral blood flow [13].

Astrocytes are star shaped cells that have many protrusions from their cell bodies and interact with the EC by wrapping their end feet-like processes around the vessels. Often the entire surface of the vessel is covered by the astrocyte feet. Moreover, a single astrocyte may be in contact with multiple capillaries. They participate in both the formation and maintenance of the BBB [14]. Furthermore, they also play a role in the ion, water and neurotransmitter regulation of the brain [12]. The integrity of these cells is maintained by the basement membrane which is comprised of an ECM that is produced by astrocytes, pericytes and endothelial cells and consists of type IV collagen, laminin, nidogen an perlecan. Besides providing integrity for the BBB, the membrane also functions as an intercellular communication regulator [11].

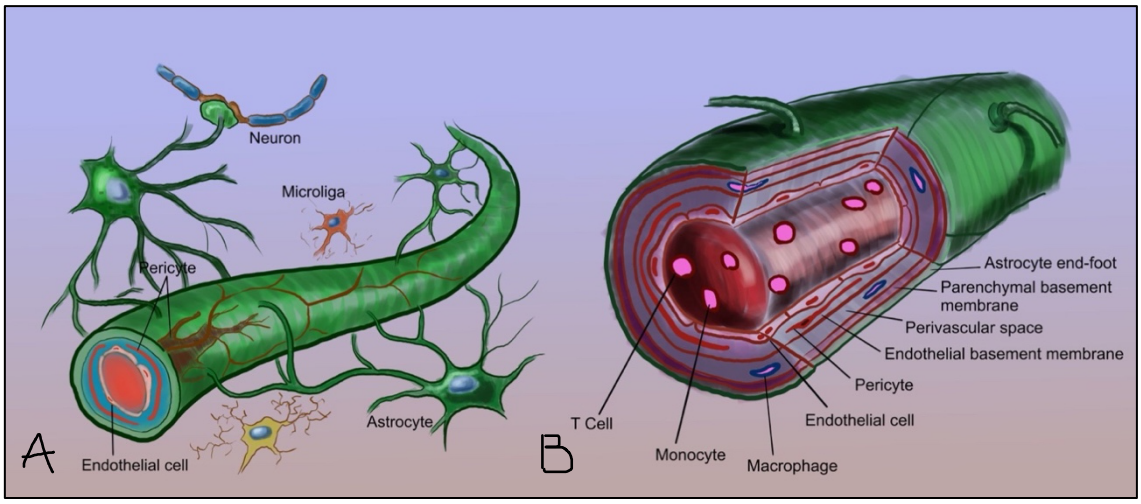

Figure 1. Structure of the NVU. The components of the neurovascular unit (NVU). The main structures are the endothelial cells that form the blood vessel wall and are closed by interendothelial junctions. The abluminal side of the endothelium and pericytes is covered by the basement membrane. Illustrated in picture A are processes extended by pericytes along the vessel wall. The astrocyte foot processes are also shown on the abluminal side of the vessel. Neurons and microglia are also depicted as they too are part of the NVU. Picture B shows a cross-section of all layers of the BBB. This figure was redrawn by the author of this review. Original figure source: [11].

2.2 Blood brain barrier permeation mechanisms

There are two main ways for molecules to pass the BBB and reach their target tissues. One of these ways is the paracellular path which is severely restricted due to the reported 1.4 – 1.8 nm intercellular gap sizes. Nonetheless, particles with a size smaller than 1 nm may be transported using this method through water filled intercellular spaces called pores. The second way is through transcytosis. This implies that the molecules enter and subsequently exit the barrier. Paths such as passive diffusion, carrier mediated transport and receptor mediated transcytosis all fall in this category [15] together with adsorption mediated transcytosis and active efflux transporters.

Generally speaking, passive diffusion is limited to the passage of gaseous molecules such as oxygen or carbon dioxide or molecules that are lipophilic enough to cross the membrane lipid bilayer. Barbiturates, ethanol and caffeine can all pass through the barrier [14]. An approximation of the permeability of the BBB for certain molecules can be made based on the molecule's octanol/water partition coefficients. Drugs with a high partition coefficient such as diphenhydramine can easily pass the barrier [1]. Factors restricting the passage of molecules include a high polar surface area, a tendency to form more than six hydrogen bonds, rotatable bonds and a weight above 450 Da [16]. Carrier mediated transport presents a wide range of carriers for molecules such as glucose (GLUT-1),

lactate and pyruvate (MCT1), creatine (CrT) and large amino acids (LAT1) to be passed through the BBB. On the other hand, molecules such as insulin, leptin and transferrin are transported through receptor mediated transport [14] where ligands bind a specific receptor and undergo an endocytic pathway [15]. Large molecules such as albumin or histone use adsorption mediated transcytosis. Finally active efflux transporters include P-glycoprotein, BCRP [14].

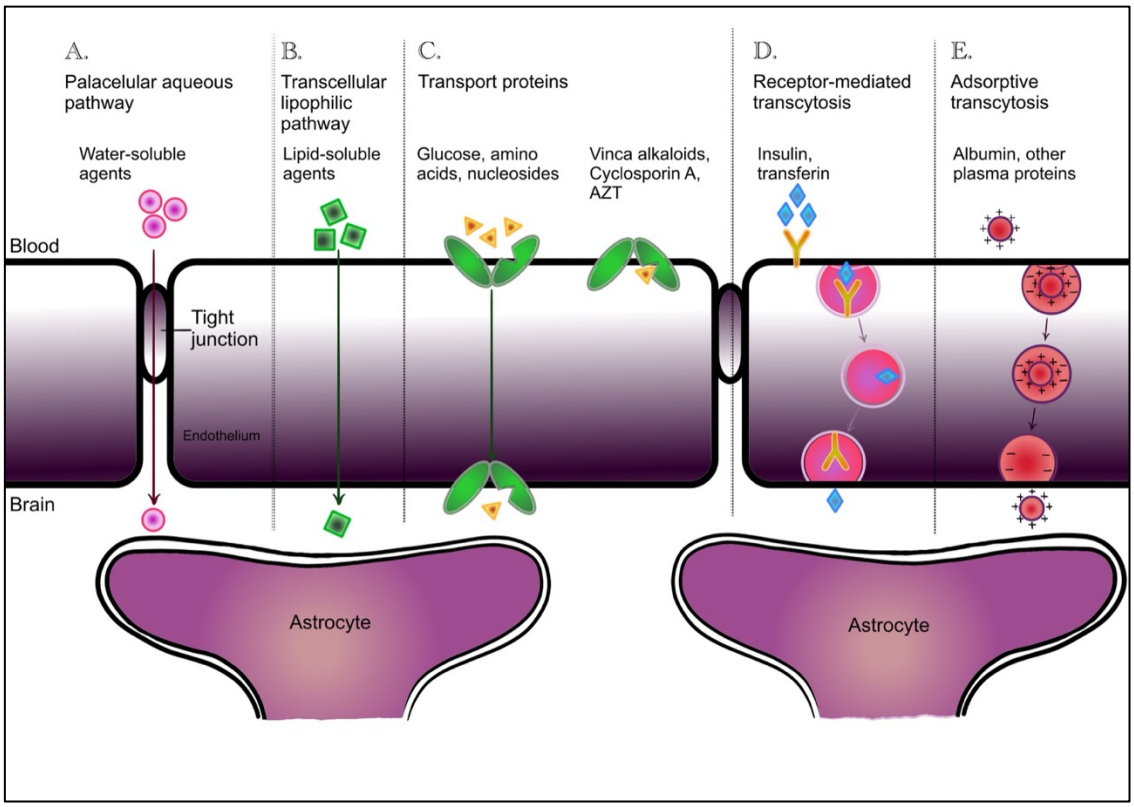

Figure 2. Main molecular ways of crossing the BBB. The picture shows a diagram of the BBB and the astrocyte end-feet. The main molecular access ways across the BBB are depicted. **A.** Passage of water-soluble molecules is reduced drastically by tight junctions. **B.** The endothelial lipid membrane grants access to several lipid-soluble molecules. **C.** Transport proteins are shown for compounds such as glucose, amino acids and nucleosides some of which are energy dependent (AZT). **D.** Molecules like insulin and transferrin cross by receptor-mediated endocytosis and transcytosis. **E.** Proteins like albumin undergo increased uptake through adsorptive-mediated endocytosis and transcytosis if cationized. This figure was redrawn by the author of this review. Original figure source: [12].

2.3 The blood brain barrier in drug delivery

Although the BBB protects the brain from a multitude of insults such as pathogens, inflammation and toxins [6] it also greatly inhibits the passage of most modern drugs. All available modern drugs that consist of large molecules are inhibited. In addition, a

reported 98% of small molecules are also blocked from crossing [2]. Current strategies for drug delivery include both invasive and non-invasive methods. The invasive methods tend to be avoided due to the high risk they carry. They include opening the tight junctions for a limited period, intracerebral drug injection and catheter guided drug injection. Non-invasive methods include the intranasal way where the drug travels along the olfactory and trigeminal neural pathways as well as the intravenous way. Due to the skill required to master intranasal administration (accidentally administering the drug into the stomach or lungs) the intravenous way is preferred. A promising technique related to IV administration is the use of focused ultrasound to open the BBB, but this has not been thoroughly extensively researched yet. Due to all the aforementioned complications, nanoparticles are viewed as a possible alternative method of crossing the BBB to deliver drugs [17]. Nanomaterial such as polymeric nanoparticles have many desirable advantages such as a systemic route of administration, protection against enzymatic degradation, high lipophilic drug solubility, controlled and sustained drug release and enhancements of drug delivery across the BBB for CNS therapy [18].

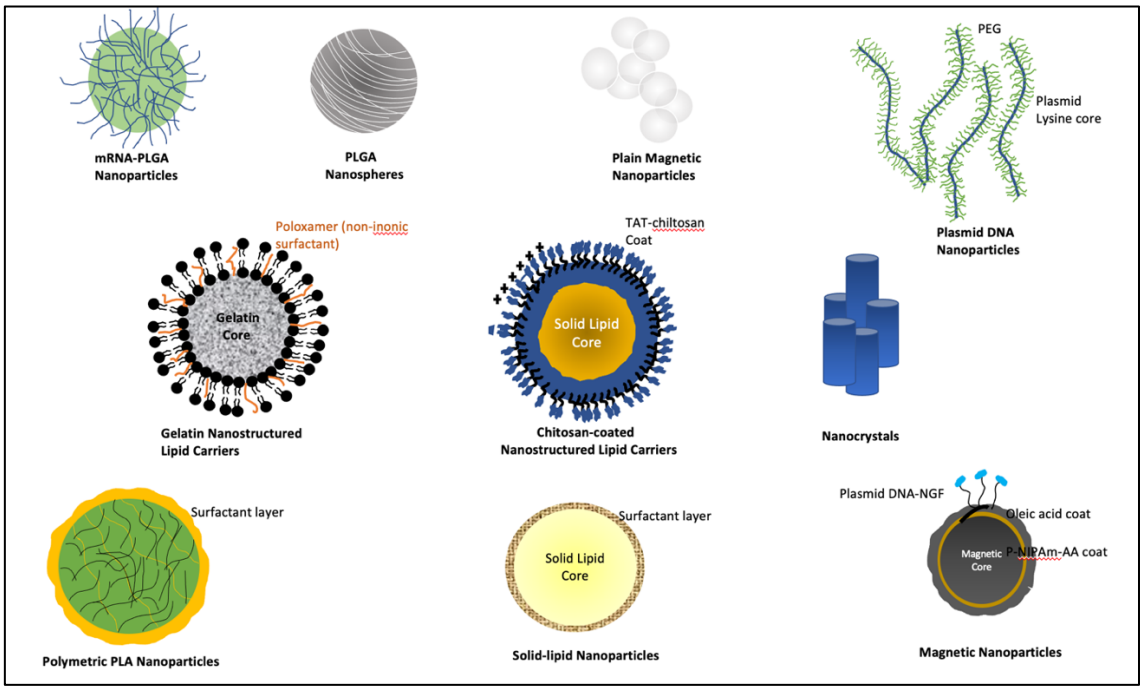

Figure 3. Nanoparticles. Some of the most researched nanoparticles recently. This figure was redrawn by the author of this review. Original figure source: [19].

3. Alzheimer's disease

Alzheimer's disease is a neurodegenerative disease that leads to slow but progressive cognitive disfunction and ultimately ends in death. It is thought that the pathophysiological processes related to Alzheimer's can start 20 years prior to the onset of symptoms. The prevalence of the disease is high compared to other neurodegenerative ailments with an estimate 5.8 million people suffering of Alzheimer's in the United States. This figure is projected to increase more than twofold until 2050 when it will reach approximately 13.8 million people due to the increasing elderly population [20].

The disease was described for the first time in 1906 by Alois Alzheimer. The patient in question was Auguste D., a 51-year-old woman at the Frankfurt Hospital who subsequently passed away 4.5 years later [21]. Clinical signs include impaired memory and recalling ability, language difficulties, disorientation, apathy, agitation, depression and delusions [22]. Even though a definitive diagnosis of the disease requires a post-mortem examination of the brain, recent advancements allow for a diagnosis accuracy of approximately 95% in living patients. This is done with the help of several tools such as history, cognitive function evaluation, neuropsychological tests, ruling out other neurodegenerative [23]. Unfortunately, differentiating Alzheimer's from the other diseases can only be possible in the advanced stages. On the bright side, developments in the imaging and biomarker areas have given researchers an invaluable understanding of Alzheimer's. MRI scanning is a good tool for studying anatomical and connectivity changes while PET scans are used to track Aβ and tau spreading and give a better understanding of the pathophysiology of the disease [24].

3.1 Alzheimer's disease pathogenesis

Although still controversial, the current understanding of Alzheimer's disease revolves around the amyloid hypothesis. According to this theory, the pathogenesis of Alzheimer's is linked to an overproduction or decreased clearance of Aβ peptides which lead to plaque formation in the brain tissue. This results in inflammatory cascades, increased oxidative stress, mitochondrial dysfunction, apoptosis, hyperphosphorylation of Tau protein and other homeostatic disturbances that end in neuronal death. Controversy stems from the fact that studies have shown that non-demented elderly individuals also exhibited plaque

formation. Moreover, anti- amyloid treatments failed to improve cognitive function [25]. The pathogenesis starts with a type 1 transmembrane protein called amyloid precursor protein (APP) which can be cleaved to serve as a source for two different pathways. In the first pathway, the nonamyloidogenic pathways, APP is cleaved by α-secretase resulting in sAPPα and an 83 amino acid fragment called C83. Finally, C83 can be further cleaved by γ-secretase to form the product p3. The second pathway, the amyloidogenic pathway, commences with APP being cleaved by β-secretase to form sAPPβ and C99. The latter is then cleaved by γ-secretase as well to form Aβ. Recent studies suggest that both Aβ and tau may undergo refolding and reach a subsequent infectious state. This prion-like behaviour has been termed 'templating' [26]. It is of importance to note that the length of Aβ is variable and it can range from 37 to 42 amino acids. This is relevant because the longer products are more likely to aggregate and form plaque [27]. Aβs are originally released as monomers, then gradually they aggregate into dimers, octamers, proto fibrils, fibrils and eventually plaque [25]. These events result in neuronal death and compromised acetyl-choline function [27].

The second culprit of Alzheimer's disease is protein Tau. Tau proteins are present in many species from rodents to humans and play a crucial role in neuronal microtubule formation [28]. The microtubules are rigid cellular structures that give and maintain the shape of the cells. In addition, they act as motorways for molecules bound to protein transporters, thus they need to be straight [29]. Tau stabilizes the microtubule shape by interacting with α- and β-tubulin through phosphorylation and dephosphorylation, therefore; it can be said that this process is a dynamic one [25]. In Alzheimer's disease tau is abnormally hyperphosphorylated which means that it cannot bind to the microtubules anymore and results in tangled filaments [28].

3.2 Polymeric nanoparticles

Nanoparticles favorable for drug delivery and therapy of Alzheimer's disease can fall in categories such as: polymeric nanoparticles, liposomes, metallic nanoparticles and cyclodextrins. Polymeric nanoparticles range in size from 1 to 100 nm and are usually composed of biodegradable and biocompatible PLGA (poly lactic-co-glycolic acid), PLA (polylactic acid) or PBCA (poly butyl cyanocrylate) for example. The most researched of

these is PLGA, which is degraded through hydrolysis and eliminated by physiological pathways [17].

Curcumin encapsulated PLGA NPs are the central subject of various studies that claim they could be used as a novel therapy for Alzheimer's disease. Curcumin is a compound extracted from the rhizome of the *Curcuma longa* plant and it is known for its anti-inflammatory properties. Studies have shown that curcumin NPs may reduce inflammation, induce neurogenesis and decrease Aβ production. Cur-PLGA NPs were shown to reverse learning and memory decline in a rat model with induced beta amyloidosis [30]. When compared to curcumin alone, Cur-PLGA was demonstrated to increase the bioavailability at least 9 times. Another study found that adding PEG (polyethylene glycol) also increased the bioavailability of curcumin. With this in mind, a new nanomaterial was developed, namely, Cur-loaded PLGA-PEG-B6 particles. B6 peptide was chosen due to its potential enhancement of BBB crossing because it originates from a phage display library that targets the transferrin receptor in the BBB. This receptor has been studied intensely for BBB targeting. Mice testing of Cur-loaded PLGA-PEG-B6 was done using the Morris water maze. Mice treated with the nanomaterial crossed the platform faster and spent less time searching for the platform. Therefore, these mice had improved memory and spatial learning capacity [31]. [32] found that conjugating curcumin loaded PLGA NPs with Tet-1 peptide did not affect the properties of curcumin and greatly increased neuron targeting. No *in vivo* research was conducted in this case. Another study conducted by [33] used a similar approach of conjugating curcumin with a peptide. Here, curcumin encapsulated PLGA was conjugated with g7 peptide. *In vitro* results showed that neurons treated with Cur-PLGA-g7 exhibited partial restored health. Besides this, increased levels of the nanomaterial did not lead to cell toxicity which entails that this is a promising therapy method for Alzheimer's disease. Different studies suggest that CRT conjugated PLGA nanoparticles loaded with Aβ generation inhibitor S1 and curcumin enhance BBB passage of the nanomaterial and reduce the number of activated glial cells [30].

One study conducted in 2016 loaded PLGA NPs with Quercetin (PLGA@QT NPs). It is an antioxidant found in many fruits and vegetables that has been proposed to have the ability to reduce amyloid toxicity. When tested on rats with the Morris water maze and the Novel Object Recognition test. The Novel Object Recognition test is based on the

natural inclination of animals to interact more with a new object as opposed to a familiar one. The PLGA@QT NP animals displayed improve cognitive abilities. Moreover, Aβ42 aggregation was decreased by the nanomaterial [34].

Galantamine is a reversible acetylcholine esterase inhibitor that can be used to lessen he cognitive disfunctions present in Alzheimer's disease. Furthermore, some studies showed that the drug also leads serotonin, glutamate and dopamine release which could also aid Alzheimer's patients [35]. Galantamine loaded PLGA nanoparticles have been prepared with an encapsulation efficacy of 98%. However, therapeutic doses could not be reached so there is need for a concentration step before *in vivo* IV administrations. The release of the drug is comparable to micellar or aqueous solutions and pharmacological activity of the encapsulated material remains at 80% [18]. Dexibuprofen loaded PLGA NPs have been studied with the goal of increasing the therapeutic effect of dexibuprofen through crossing then BBB [17]. Dexibuprofen is a non-steroidal anti-inflammatory drug and a more pharmacologically potent and active enantiomer of ibuprofen [36]. The dexibuprofen loaded nanomaterial proved to be nontoxic and have a higher passage rate of the BBB after oral gavage of mice. This is also viewed as a promising candidate for Alzheimer's treatment. Phytol loaded PLGA NPs have also been prepared and resulted in improved efficacy compared to donepezil. However, it is not known yet if these NPs can cross the BBB *in vivo* [17]. A different study analyzed the possibility of using poly-amidoamine (PAMAM) dendrimers in drug delivery across the BBB. A dendrimer is a highly branched, 3-dimensional polymeric nanoparticle. In order to avoid *in vivo* toxicity, the dendrimers were kept at 10% amine surface groups by replacing surface amine groups with hydroxyl (-OH) moieties. Both the *in vitro* and the *in vivo* studies prove that these modified PAMAM particles could cross the BBB and be a potential candidate for drug delivery. The study used twenty-one mice that were injected with the nanomaterial through the carotid artery [37].

Nanoparticles were also created using chitosan, a chitin derivative. Chitosan has received significant attention in the medical community lately due to its exceptionally low immunogenicity, low toxicity, biodegradability and biocompatibility [38]. Thus, in 2020 chitosan nanoparticles were prepared and loaded with sitagliptin. The drug is usually used in the treatment of diabetic patients, but it was chosen for the study due to reported similarities between Alzheimer's disease and type-2 diabetes mellitus and sitagliptin

being able to reduce symptoms of Alzheimer's. The study found that an IN application of the chitosan nanomaterial increased the drug's concentration in the brain [39]. An earlier study loaded chitosan NPs with piperine and used the IN route for administration. The formulation was tested against the standard drug, Donepezil which was administered intraperitoneally, and the rats were tested on multiple occasions during the experiment by using the Morris water maze. The results suggested that Donepezil and the piperine NPs administered IN gave similar results. They both improved the cognitive function of the animals. It is important to state that piprine is a known irritant of the mucosa. When tested on lab rats, 85% of the animals showed signs of irritation such as sneezing and nose rubbing. No such side effects were observed with the chitosan NPs because of their ability to encapsulate piperine and prevent its direct contact with the mucosa [40].

3.3 Liposomes

The possibility of delivering drugs across the BBB using liposomes as nanoparticles is also explored. Natural lipids such as lecithin, sphingomyelin and phosphatidylcholine or synthetic compounds like 1,2-dipalmitoyl-sn-glycero-3-phosphocholine and ethyl-phosphatidylcholine can be used in liposome production [41]. The possibility of the liposome's crossing the BBB may be due to their bilipid layer. However, modifications to the liposomes are needed to ensure a more competent crossing [42]. Therefore, most used in Alzheimer's disease are the PEGylated 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-PEG 2000. The preferred size to cross the BBB is 100nm or below even though this means that the drug load is relatively small [41]. PEGylated curcumin nanoliposomes were used for delivery across the BBB. They were able to inhibit Aβ peptide aggregation during the entire course of this study. Furthermore, their ability of targeting the brain was only slightly affected by the curcumin load. An *in vitro* model of the BBB was used to demonstrate this [43].

Rivastigmine is an FDA approved pseudo-irreversible acetylcholine esterase and butyrylcholine esterase inhibitor used for therapy of mild to moderate Alzheimer's disease [44]. The drug is limited from a stability point of view as well as permeation through the BBB [41]. CPPs (cell penetrating peptide) have been selected for enhancing BBB permeability due to their ability to cross the cell membrane through endocytosis and transcytosis [42]. As such, the possibility of modified DSPE-PEG-CPP liposomes

delivering rivastigmine through the IN route was studied. It is currently believed that drugs can reach the brain via the IN route in two ways. Either by the olfactory route or by crossing the BBB. Results show that CPP modified liposomes were more efficient at delivering rivastigmine than regular rivastigmine loaded liposomes. The IN route was also demonstrated to be more efficient than the IV route [45]. A different study found that this type of administration increased drug delivery four-fold [46].

Quercetin has also been loaded in lipid nanoparticles. This study used both SLN (solid lipid nanoparticle) and NLC (nanostructural lipid carrier) particles functionalized with transferrin. A cell monolayer similar to the BBB was used to assess the permeability of the particles. NLC performed better than the SLNs for both functionalized and non-functionalized particle types. The study also proved *in vitro* that the quercetin NPs were able to reduce Aβ (1-42) aggregation. The most efficient of the tested formulations were the transferrin functionalized NLCs. Thus, the study concluded that this approach could improve the treatment of Alzheimer's disease [47]. SLNs have also been studied when loaded with nicotinamide. Although nicotinamide can readily cross the BBB, very high doses are required due to sink conditions. The aim of the study was to synthetize a formulation that could deliver optimal doses of nicotinamide to the brain and possibly reduce the dose and administration time of the drug. The results showed the rats injected with the nicotinamide PS-SLNs (phosphatidylserine SLNs) displayed improved memory and reduced amount of tau hyperphosphorylation [48].

3.4 Metallic nanoparticles

Metallic nanoparticles such as selenium have also been researched in hopes of aiding Alzheimer's patients. Metal particles have a size between 1 and 200 nm [49]. Selenium is of particular interest due to its high antioxidant capabilities that can reduce oxidative stress and Aβ aggregation [50]. Sialic acid modified selenium nanoparticles covered with B6 peptide have been proven to cross the BBB *in vitro.* Moreover, they led to a decrease in Aβ aggregation [51]. An earlier study made use of EGCG (Epigallocatechin-3-gallate) selenium NPs. Epigallocatechin-3-gallate is an antioxidant found in green tea. Several studies report that EGCG interacts with amyloid protein such as Aβ, thus it could show considerable relevance in the case of Alzheimer's treatment. The study proved that EGCG-SeNPs were able break down Aβ fibrils into low toxicity compounds [52]. The

promising features of selenium NPs have been further consolidated in a recent study that prepared chitosan and cholinergic acid SeNPs (CGA@ChSeNPs). Not only did these NPs hinder Aβ aggregation but they proved to have exceptional antioxidant abilities [53].

Similarly, gold NPs (AuNPs) also have antioxidant and anti-inflammatory traits that can be useful in neurodegenerative diseases. Wistar male rats have been used to test AuNPs *in vivo.* The animals were treated with the nanomaterial by intraperitoneal injection. Subsequently they were tested using the Barnes maze and killed 24 hours later. The selected cortical areas of study were the pre-frontal cortex and the hippocampus. Analysis demonstrated that AuNPs inhibited tau protein hyperphosphorylation in both areas[54]. Polyoxometalate with Wells Dawson structure peptide gold particles (AuNP POMD-pep) have also been successfully used to cross the BBB and inhibit Aβ aggregation [55]. Ceria NPs with a size smaller than 5nm have also been tested for the purpose of improving Alzheimer's therapy. This study revolved around the notion that mitochondria play a central role in Alzheimer's disease pathogenesis. TPP-ceria treated 5XFAD mice showed less astrocyte and microglia inflammatory processes. They were able to protect the mitochondria by reducing ROS levels. However, these NPs did not show the capacity of eliminating Aβ plaques [56].

3.5 Cyclodextrins

Cyclodextrins are cyclic oligosaccharides first discovered in 1891 [57] the inner cavity of which can hold lipophilic molecules [17]. For example, β-cyclodextrin is composed of seven glucose units that allow it to form a hydrophobic inner chamber and a hydrophilic outer surface. It is a non-degradable compound that is excreted via the kidney following IV administration. The authors of a 2012 study conducted the synthesis of CD-p-AE (β-cyclodextrin-poly (β-amino ester)) nanoparticles and elegantly proved *in vitro* that these nanoparticles did not disrupt the integrity of the BBB and had very high permeability coefficients. The drug chosen to be loaded in these particles in this study was doxorubicin. The study found that release of doxorubicin lasted one month [58]. A different study used the hydroxypropyl form of the β-CD in mice overexpressing APP. Subcutaneous injections were administered to these mice from day 7 to the age of 4 months. Results of this study show that Aβ plaque formation was notably reduced in the treated mice. A

second significant finding revolved around the fact that the mice has improved memory in the Morris water maze test [59].

Nanosystem	**Load**	**Result**
PLGA	Curcumin	Reversed learning and memory deficit
PLGA-PEG-B6	Curcumin	Improved memory and spatial learning
Tet-1 conjugated PLGA	Curcumin	Increased neuron targeting
G7 conjugated PLGA	Curcumin	Partial restored health
CRT conjugated PLGA	Curcumin+ Aβ generation inhibitor S1	Increased BBB crossing
PLGA	Quercetin	Increased cognitive ability Decreased Aβ plaque
PLGA	Galantamine	No therapeutic dose achieved
PLGA	Dexibuprofen	Increased delivery across the BBB
PLGA	Phytol	Improved efficacy over donepezil
PAMAM dendrimer	-	Ability to cross BBB
Chitosan	Sitagliptin	Increased brain drug concentration
Chitosan	Piperine	Improved cognitive function
PEGylated liposome	Curcumin	Aβ aggregation inhibition
DSPE-PEG-CPP liposome	Rivastigmine	Increased delivery
SLN	Quercetin	Decreased Aβ aggregation
NLC	Quercetin	Decreased Aβ aggregation
PS-SLN	Nicotinamide	Improved memory Reduced tau hyperphosphorylation
Sialic acid modified Se NPs covered with B6	-	Decreased Aβ aggregation

Se	EGCG	Broke down Aβ fibrils
CGA@ChSe	-	Reduced Aβ aggregation Excellent antioxidant abilities
Au	-	Inhibited tau hyperphosphorylation
AuNP POMD-pep	-	Inhibited Aβ aggregation
TPP-ceria	-	Reduced ROS levels
CD-p-AE	Doxorubicin	Crossed BBB efficiently
Hydroxypropyl β-CD	-	Improved memory Reduced Aβ aggregation

Table 1. *Reviewed nanosystems used to improve treatment of Alzheimer's disease, their load and the result of the study.*

4. Parkinson's disease

Parkinson's disease is ranked as the second most common neurodegenerative disease in the geriatric population of the United States [60]. Approximately 1 million Americans and 4 million people worldwide are affected by the disease. The prevalence of the disease is much higher in the older population with cases in people under 40 years of age rarely occurring. It is also thought that two times more men develop the disease compared to women [61].

4.1 Pathogenesis of Parkinson's disease

The disease was first described two hundred years ago, in 1817, by James Parkinson in his paper 'An essay on the shaking palsy'. Parkinson described six different cases consisting of males that reported the first symptoms of the disease between the ages of 50 and 65 [62]. The cause of Parkinson's disease is attribute to a complex combination of genetic and environmental factors [63] that leads to the loss or degeneration of neurons that produce dopamine (dopaminergic) in the substantia nigra and the formation of Lewy bodies in these neurons. It is crucial to note that pathological changes may occur more than 20 years before any clinical signs [60]. The clinical presentation of the disease revolves around motor and non-motor symptoms. Motor symptoms include tremor, rigidity and bradykinesia. On the other hand, non-motor symptoms include sleep disturbances, sensory symptoms and cognitive dysfunctions [64]. Diagnosis is based on the presence of the typical features of Parkinson's. If the patient reports a gradual manifestation of the previously mentioned features and responds positively to levodopa treatment, Parkinson's disease is the most likely culprit [60].

4.2 Polymeric nanoparticles

The most efficient treatment of Parkinson's disease currently is treatment with levodopa which is converted into dopamine inside the body [65]. Thus, dopamine loaded PLGA NPs have been tested to study the possibility of crossing dopamine over the BBB for Parkinson's treatment. The prepared nanomaterial was tested for oxidation. It was found that the dopamine present within the dopamine loaded NPs (DA NPs) was less available to oxidation than bulk dopamine. The particles were further tested for toxicity and it was found that they are less cytotoxic than bulk dopamine. The *in vivo* part of the study

demonstrated that DA NPs administered systemically through IV infusion were able to improve motor dysfunction in the tested rats. Furthermore, no adverse effects on the dopaminergic system were observed and cytotoxic effects on non-targeted tissues was absent [66]. Similarly, L-DOPA (L-3-4-Dihydroxyalanine) loaded PLGA nanoparticles were also tested *in vivo* through IN administration for the purpose of Parkinson's treatment. The particles were tested against standard drugs. The study found that all of the drugs were able to improve Parkinson's features in the tested rats after one administration. However, a week later, there was a notable decrease in the effect elicited by the standard drugs. On the other hand, the nanomaterial's effect improved. Animals were tested multiple times using the placing test, the vertical grid holding test, the open field test and the footfault asymmetry test. The conclusion of the study found that the created nanoparticles restored the motor function and had a long-lasting effect in the rat model. Subsequently, this may lead to a decreased administration frequency and diminished drug dose in human patients [67].

A study conducted by [68] used PEGylated PLGA NPs conjugated with lactoferrin and loaded with urocortin to investigate the possibility of Parkinson's treatment. Lactoferrin, a protein belonging to the transferrin family, was chosen since lactoferrin receptor can be found on the BBB. Lesions were induced using 6-hydroxydopamine (6-OHDA). The study proved for the first time that urocortin, which was previously proven to hinder Parkinsonian-like features through invasive administration, could be delivered non-invasively by using lactoferrin NPs. A similar earlier study carried out by [69] used modified lactoferrin NPs loaded with human GDNF to improve Parkinson's relate dysfunctions. This time, the authors used PEGylated PAMAM particles as a means of delivery. Results showed that injections with the modified particles via the tail vein led to better locomotor activity and decreased loss of dopamine producing neurons. Is it important to note that in all of the three previously mentioned studies lesions were induced by using 6-hydroxydopamine. Another study done by [70] used IN administered lactoferrin modified PEG-PLGA particles loaded with rotigotine for brain delivery. The modified particles exhibited low level of cytotoxicity and improved cellular accumulation when compared to regular NPs. Delivery of rotigotine was also enhanced by using the modified material. A recent study loaded rotigotine in chitosan nanoparticles for nose-to-brain delivery. The results suggested that this approach could be more advantageous than the conventional method [71].

Selegiline, an irreversible MAO inhibitor used to treat early signs of Parkinson's has also been loaded into PLGA to form SGN-NPs. This study tested the efficacy of transdermal patches containing the selegiline NPs. The patches were evaluated for erythema and edema formation, and it was concluded that no signs of irritation were present 24 hours later. Mild redness of the skin was present after 48 and 72 hours. It was found that compared to IV administration the transdermal patch method presented delayed uptake of the drug and a longer duration of action which makes it ideal for progressive neurodegenerative diseases such as Parkinson's. Moreover, a transdermal application is non-invasive and elderly friendly. The method resulted in increased dopamine levels and reduced catalepsy [72]. A 2020 study conducted by the same authors also exploited the idea of rasagiline mesylate being administered the same way. This study also concluded transdermal patches containing rasagiline NPs could represent a novel treatment method for Parkinson's due to its brain targeting capacity, prolonged release an non-invasive nature [73].

Another study loaded Pramipexole, a dopamine agonist usually used in combination with levodopa to treat Parkinson's disease, in chitosan nanoparticles for IN administration. The formulation was tested against an intranasal drug solution and a tablet dosage. The study concluded that the chitosan particles loaded with pramipexole performed the best out of the three, especially in terms of brain dopamine levels [74].

4.3 Lipid nanoparticles

Solid lipid nanoparticles (SLN) such as triglycerides also show potential. Moreover, due to their small size they may be used in parenteral administration. The drug of choice for this study's approach was bromocriptine [75]. Bromocriptine is an ergot alkaloid that exerts a dopamine agonist effect. Although superior to levodopa in hormonal disease treatment, the same cannot be said in case of Parkinson's [76]. Although the efficacy of free bromocriptine and NLC encapsulated bromocriptine were the same, the encapsulated drug had a longer half-life than its free counterpart [75]. Apomorphine, another dopamine agonist, was also loaded in SLN nanoparticles with the intention of enteral administration. Standard apomorphine cannot be given orally due to its sensitivity to gastric contents. Thus, the NPs have been used with the intention of bypassing this deficit. They were

administered to rats through an oral intubation cannula. The study found that apomorphine loaded SLNs did increase the oral bioavailability of the drug and did increase its capability of treating Parkinson's [77]. Apomorphine NLCs have also been used by [78]. NLCs avoid the storage problems that SLNs are subject to. According to the findings of this study brain targeting was notably improved.

Ropinirole was used to load both NLCs and SNLs in a recent study. Ropinirole is a newer generation dopamine agonist drug that can be used both alone and in combination with levodopa to lower the second drug's dose [79]. The difference between NLCs and SLNs lies in their structure. While SLNs are soli lipids, NLCs are composed of a solid matrix that contains liquid lipid compartments [78]. The ropinirole NLC and SNL formulations proved to be stable and easily manufactured. Results pointed towards an increased oral and transdermal bioavailability. Furthermore, the transdermal preparation proved to be safe after being tested and carefully observed [79]. Ropinirole hydrochloride has been loaded in polymer-lipid hybrid nanoparticles (PLN). The chosen route of administration in this study was IN. Therapeutic capacity was similar to the standard oral formulations hence lowering the dose and frequency of administration [80].

Dual loaded curcumin and piperine glyceryl monooleate (GMO) nanoparticles have been studied as well. Both curcumin and piperine therapeutic doses are hard to deliver to the brain due to their low permeability of the BBB. The results of the study show that curcumin oral bioavailability was increased by this particular delivery method. Secondly, the drugs managed to cross the BBB and improve the motor coordination of the animals [81].

4.4 Metallic nanoparticles

Cerium oxide NPs have also been examined as a possible treatment method for Parkinson's in a study done by [82]. The rats used in the study underwent intrastriatal injections of 6-OHDA to cause parkinsonian lesions. They were subsequently treated intraperitoneally with the cerium oxide NPs (CeO2NPs) for three weeks and tested using several different tests such as open field, rota rod or the stepping test. The effects elicited by the nanomaterial varied with the best results coming from a 0.5mg/kg dose. The rats treated with this dose were the closest to the control group in terms of behavior. Although

further research is needed, the study concludes that cerium oxide NPs can increase striatal dopamine levels through their antiapoptotic and antioxidant nature. Similarly, in a recent study, [83] used cerium oxide NPs in a yeast model of Parkinson's. The particles were able to reduce α-Sinuclein toxicity.

Liu et al. (2020) developed a new gold-based nanomaterial with the aim of studying Parkinson's therapy. The gold nanoparticles (AuNPs) were linked to plasmid DNA through electrostatic adsorption. The AuNPs (pDNA) were further encapsulated in liposomes. Next, NGF (nerve growth factor) targeting molecules were grafted on these particles. Finally, DHA (docosahexaenoic acid) was attached to the particles resulting in AuNPs-pDNA-Lipo-NGF-DHA NPs. The results show that these NPs protected dopaminergic neurons *in vivo*. Both spatial memory and bradykinesia were improved.

Nanosystem	**Load**	**Result**
PLGA	Dopamine	Improved motor function
PLGA	L-DOPA	Improved motor function Longer lasting effect
Lactoferrin conjugated PEGylated PLGA	Urocortin	Urocortin delivered non-invasively
Lactoferrin modified PEGylated PAMAM	GDNF	Improved motor function
Lactoferrin modified PEG-PLGA	Rotigotine	Enhanced rotigotine delivery
Chitosan	Rotigotine	Improved over conventional methods
PLGA	Selegiline	Increase dopamine levels Reduced catalepsy
PLGA	Rasagiline mesylate	Prolonged release
Chitosan	Pramipexole	Increased dopamine levels
NLC	Bromocriptine	Longer half-life
SLN	Apomorphine	Increased bioavailability
NLC	Apomorphine	Improved brain targeting
SLN, NLC	Ropinirole	Increased bioavailability

PLN	Ropinirole hydrochloride	Lowered dose and frequency
GMO	Curcumin, piperine	Improved motor function
Cerium oxide	-	Increased dopamine levels
Cerium oxide	-	Reduced α-Sinuclein toxicity
AuNPs-pDNA-Lipo-NGF-DHA	-	Improved spatial memory and bradykinesia

Table 2. *Reviewed nanosystems used to improve treatment of Parkinson's disease, their load and the result of the study.*

5. Huntington's disease

Huntington's disease can be characterized as a progressive neurodegenerative, autosomal-dominant disease that leads to unpredictable muscular movement, cognitive and behavioral disorders. Symptoms of Huntington's can arise in all individuals aged between 1 and 80 [84]. The prevalence of the disease is approximated at 4-10 individuals in every 100 000 in the western world. The clinical manifestation of the disease can usually be seen around the age of 40 with subsequent death 15 to 20 years later [85]. It is noteworthy that the prevalence seems to be lower in those with non-European ancestry.

5.1 Pathogenesis of Huntington's disease

The primary abnormality that leads to Huntington's is caused by abnormal glutamine coding trinucleotide repeats in the huntingtin protein. Sequences of cytosine, adenine and guanine (CAG) are responsible for this increase. Healthy individuals have anywhere from 9 to 29 CAG repeats whereas affected patients usually have 39 or more repeats [86]. Huntingtin is a large molecule composed of HEAT repeats of 50 amino acids [85]. Although a wide range of structures are affected in Huntington's, the most characteristic neuropathological lesion of the disease is the loss of neurons in the cortex and striatum [87].

The clinical manifestation of the disease can be divided into three main parts, namely, motor, cognitive and behavioral. The motor dysfunctions include mostly chorea, dystonia, bradykinesia and apraxia. This leads to recurrent falls which increase the mortality of the disease. The end-stages of the disease tend to be characterized by increased dystonia, dysphagia and dysarthria. From a cognitive point of view, the disease manifests itself as subcortical dementia. The behavior of the individual is negatively impacted as well. The patients manifest suicidal thoughts and attempts, aggression, psychosis, depression and anxiety [88]. One study mentions that around a quarter of individuals attempt suicide during the course of the disease [84].

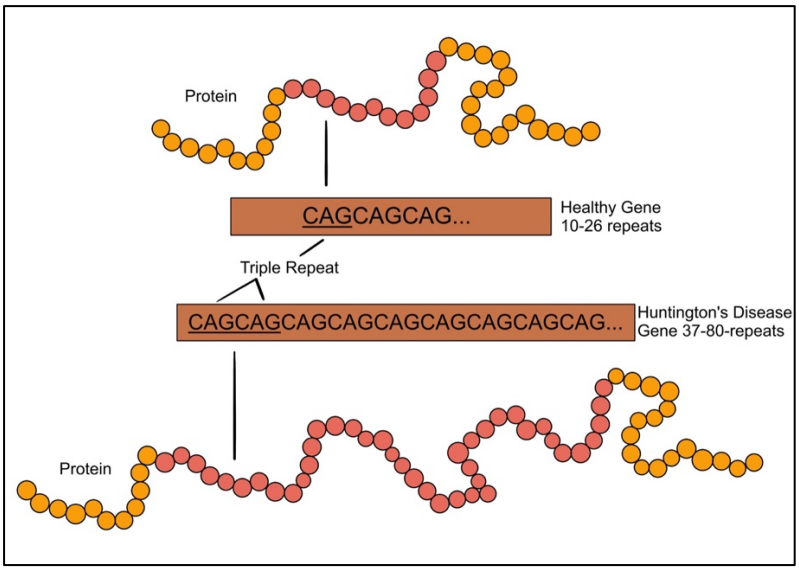

Figure 4. Huntington's disease repeats. Diagram depicting the normal range of repeats and the abnormal range of repeats found in Huntington's disease. This figure was redrawn by the author of this review. Original figure source: [89]

5.2 Polymeric nanoparticles

Polymeric NPs such as PLGA particles modified by using the g-7 glycopeptide and loaded with cholesterol have also been studied in regard to Huntington's treatment. Cholesterol was chosen as the load due to Huntington's being associated with a decrease in brain cholesterol. The study found that modifying the NPs with g-7 protein leads to increased BBB permeability and drug release. It was also stated that the treatment with these particles led to preventing impaired cognitive abilities and improved synaptic communication [90].

Chitosan nanoparticles loaded with siRNA have also been studied for the purpose of administering anti-HTT. The *in vivo* administration was done via the IN route by using two doses. A 1.2 nmol and a 5.8 nmol dose were used. The higher dose proved to be more efficient at lowering HTT. The study proved that the chitosan NPs were able to protect the payload thus lowering HTT expression. Chitosan was chosen due to its biodegradable nature namely the fact that it can be digested by lysozymes [91]. Viral vectors have been previously examined but there are concerns revolving around the immune reactions they could elicit. Cationic liposomes have also been tested *in vitro* but they have a high toxicity potential *in vivo* [92].

Poly(trehalose) nanoparticles were synthesized in an attempt to study their potential therapeutic effect in Huntington's. The nanomaterial is up to 10 000 times more performant than molecular trehalose. The particles were reported to have a size of 20-30 nm. Mutant huntingtin aggregation was first studied using a mouse neuroblastoma cell line. The results of this experiment show that the nanoparticles were able to inhibit aggregation of huntingtin. Next, the nanomaterial was tested *in vivo* on mice. The particles were observed to cross the BBB and target the brain to inhibit polyglutamine aggregation [93].

5.3 Cyclodextrins

Another study used modified β-cyclodextrin to target the huntingtin (HTT) gene using short interfering RNA (siRNA). The authors were interested in delivering siRNA to the brain because neurons have an increase resistance to RNAi penetration. It was found that when administered through a direct brain injection, these particles led to an approximative 85% HTT gene silencing after only 4 hours. These effects lasted at least seven days. It is important to note that the particles did not manage to improve grip strength, clasping behaviour and spontaneous locomotor activity. Although rapid deterioration of the animals was noted after treatment cessation this could possibly be attributed to an accumulation of HTT gene in untargeted brain areas [94].

5.4 Lipid nanoparticles

Curcumin has been studied as a possible therapeutic alternative in case of Huntington's as well. Curcumin encapsulated solid lipid nanoparticles were tested against a 3-nitropropionic acid (3-NP) induced model of Huntington's. The experiment consisted of oral administration of the nanomaterial one hour after the intraperitoneal 3-NP injection for seven days. The results of the study suggest that the curcumin nanoparticles were able to aid oxidative stress damage induced by the 3-NP by eliciting the activation of the Nrf2 antioxidant path. Furthermore, the nano preparation has been proven to increase bioavailability of curcumin [95]. A different study experimented with rosmarinic acid SLNs administered to rats through the IN route. The nasal route is often preferred due to its non-invasive nature and avoidance of possible unwanted distribution and metabolism of the drug in different tissues. Rosmarinic acid was chosen due to its antioxidative and neuroprotective characteristics regarding dopaminergic cells. Lesions were induced using

3-NP. Subsequently, the treatment schedule was set at 14 days in which all drugs and vehicles would be administered. Behavior was evaluated through rotarod activity, locomotor activity and narrow beam test. The results show that IN administration of rosmarinic acid SLNs has improved the behavior of the experimental rats in all previously mentioned evaluation methods. Furthermore, 3-NP induced oxidative stress has also been reduced in the nanoparticle treated animals [96]. SLNs have also been loaded with thymoquinone in a different study. Thymoquinone can be found in black cumin which has traditionally been used to treat several conditions due to its anti-inflammatory and antioxidant characteristics. This study also used 3-NP to induce lesions in the experimental rats. The rats were evaluated and the study concluded that treatment with thymoquinone SLNs has potential as a possible neurodegenerative disease treatment option [97].

Another IN lipid-based approach was conducted with the use of liposomes. They were loaded with deuterium-labeled cholesterol (Chol-D6) that could be easily differentiated from native cholesterol. Even though plasma levels reached a maximum after 24 hours and declined after, brain concentration remained stable even at 72 hours. The study suggests that the Chol-D6 levels reached the highest levels in the target tissue in case of Huntington's, the striatum [98].

Nanosystem	**Load**	**Result**
g7 modified PLGA	Cholesterol	Increased BBB crossing Improved synaptic communication
Chitosan	siRNA	Lowered HTT expression
Cationic liposomes	-	toxic
Poly(trehalose)	-	Inhibited polyglutamine aggregation
Modified β-CD	siRNA	HTT gene silencing
SLN	Curcumin	Increased bioavailability of curcumin Lowered oxidative stress
SLN	Rosmarinic acid	Lowered oxidative stress

		Improved behaviour
SLN	Thymoquinone	Protected brain
Liposomes	Chol-D6	Increased brain concentration

Table 3. *Reviewed nanosystems used to improve treatment of Huntington's disease, their load and the result of the study.*

6. Stroke

Stroke has been classified as the second leading cause of human death globally, causing an estimated 5.5 million deaths yearly [99]. Roughly 87% of strokes are of ischaemic origin, meaning that there is improper blood perfusion of a certain cerebral area. The second most common type of stroke is hemorrhagic stroke, which accounts for approximately 13% of cases [100] and has a one-month mortality rate of 40% [101].

6.1 Pathophysiology of stroke

The most common causes of ischaemic stroke are related to a thromboembolic pathophysiology. The embolus usually originates from a large artery due to atherosclerosis or heart disease. However, other factors such as hypertension, diabetes mellitus, vasculitis, patent foramen ovale and hematological conditions have also been linked to ischaemic stroke [102]. Hemorrhagic stroke is caused by ruptured blood vessels that lead to blood accumulation in the brain. Causes of hemorrhagic stroke include hypertension and overuse of anticoagulants and thrombolytic agents [99]. The five most prominent risk factors of stroke in 2019 were high blood pressure, high body mass index, high fasting plasma glucose, pollution and smoking [103].

Currently, the gold standard for ischaemic stroke treatment is intravenous administration of tissue plasminogen activator to reestablish perfusion of the affected area [100]. Patients suffering of hemorrhagic stroke have their vitals monitored and they may be given mannitol to eliminate edema caused by the bleeding. In case this fails, surgery to stop the hemorrhage is required, although this option is still controversial as many patients who undergo the surgical intervention do not improve [104].

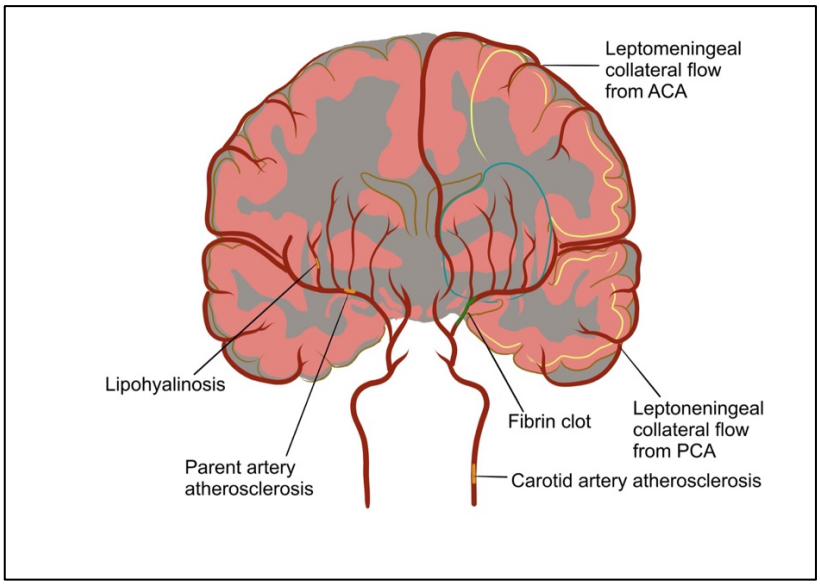

Figure 5. Ischemic stroke process. The diagram shows cardioembolic stroke, atherosclerosis of the carotid artery and smaller vessel disorders caused by lipohyalinosis and parent vessel atherosclerosis. The ischaemic core, delimited by the blue line, contains permanent damage due to a lack of collateral flow. It is surrounded by the penumbra region, delineated by the yellow line. The penumbra region is still metabolically active but lacks electrical activit. This can be reversed through reperfusion. This figure was redrawn by the author of this review. Original figure source: [102].

6.2 Polymeric nanoparticles

A study conducted in 2013 used PLGA-b-PEG nanoparticles coated with glutathione and loaded with L-3,5,3'-triiodothyronine (T3). Glutathione was chosen as a coating agent due to its capability to enhance NPs uptake in the brain. This may be due to the large amount of glutathione transporters found on the BBB. The NPs were tested on a mouse middle cerebral artery occlusion model (MCAO) of ischemic stroke. The nanomaterial showed a 58% reduction of tissue infarction and 75% reduction of edema compared to the control groups. These findings are notable because edema represents the chief reason for mortality in stroke cases. Moreover, the nanoparticle T3 formulation was able to partially protect the core of the ischemic area as well [105]. PEG-PLGA NPs have also been conjugated with wheat germ agglutinin (WGA) and loaded with NR2B9c peptide for an intranasal delivery. The reasoning for choosing WGA comes from an abundant amount of WGA receptors on the olfactory epithelium. When compared to NR2B9c or NR2B9c-NPs, the NR2B9-WGA-NPs were able to deliver more NR2B9 to the brain and improved ischemic damage [106].

In 2016 a group of researchers exploited the idea of using an autocatalytic mechanism for systemic delivery in case of stroke. The mechanism was based on PLGA particles further modified with chlorotoxin (CTX) and encapsulation of lexiscan (LEX). Lexiscan has been proven to improve BBB permeability transiently and is also approved by the FDA (U.S. Food and Drug Administration). The idea of the mechanism revolves around using CTX as a targeting ligand with high affinity for the ischaemic environment to promote the crossing of some NPs across the BBB. These particles then would release BBB modulators, LEX in this case, which would amplify the permeability of the BBB and allow for even more particles to cross over. When peptide NEP1-40 was loaded in these the PLGA-CTX/LEX NPs it was discovered that the infarct sizes were lower than the rest of the groups, and survival of the stroke mice was positively impacted [107].

Sodium hyaluronate nanoparticles have been prepared by ionic gelation methods and coated with chitosan and glycerol tripalmitin. The particles were tested by using a MCAO model and they proved to be able to cross the BBB and enter neurons. No differences were noted between the coated and the uncoated particles [108].

6.3 Liposomes

PEG-liposomes have been studied to highlight the penetration of these particles in the ischaemic area. The study was carried out on permanent middle cerebral artery occlusion model rats by injecting them with the PEG-liposomes intravenously and then monitoring the activity of the labeled PEG-liposomes by positron emission tomography (PET) for 120 minutes. Results of the PET showed that despite a significantly reduced blood flow in the area, the liposomes were able to accumulate in the ischaemic core and penumbra region [109]. PEGylated liposomes have been used earlier as well, when a different team of researchers loaded these liposomes with FK506 (Tacrolimus). The drug was chosen due to its potential of decreasing reperfusion injury. This study also made use of the MCAO model, though this time the transient variant of the model was used (t-MCAO). The liposomes were injected intravenously at the beginning of reperfusion. Subsequently animals were sacrificed 3 and 24 hours later. It was reported that the liposomes diffused solely in the ischemic hemisphere of the brain. Moreover, the liposomes displayed an antiapoptotic effect and inhibited neutrophil invasion. Importantly, administration of the free drug showed hardly any influence of the damaged area whereas administration of the

drug loaded liposomes in the same concentration showed significant improvement. Symtopms of MCAO were also alleviated in the FK506 liposome treated mice [110]. Another study focused on PEGylated liposomes conjugated with T7 peptide and stroke homing peptide (T7&SHp-P-LPs) and loaded with a novel neuroprotectant, ZL006. These dual targeting liposomes were able to deliver ZL006 successfully across the BBB and into the ischemic, affected area, thus lessening the neurological inadequacy [111].

A different study used both conventional and PEGylated liposomes loaded with tissue plasminogen activator (tPA) to prolong the half-life of tPA. Even though tPA is the best of the plasminogen activators, it has a very short half-life of 2-6 minutes. The results obtained showed a 16 to 21 fold increase in tPA half-life therefore, allowing for lower dosages and less associated risks in thrombosis treatment [112]. Accelerated thrombolysis was also demonstrated while using chitosan coated PLGA nanoparticles loaded with tPA in a clot-occluded tube model [113].

6.4 Nanoemulsions

A group of researchers studied the incorporation of curcumin in a nanoemulsion (NE) in relation to hemorrhagic stroke. As stated by the authors the nanoemulsions "are surfactant stabilized heterogeneous systems composed of oil droplets dispersed in water or aqueous medium". The study compared the performance of a nanoemulsion with 30 mg/kg curcumin termed NC30 with free curcumin 30 mg/kg termed FC30. The outcome of the study was that NC30 treated mice possessed better motor control and performed better in the open field test and the beam-walking test than the control groups. Importantly, a hematoma size 56% smaller than the control was noted. No cytotoxic effects were found against the liver and kidney [114].

6.5 Metallic nanoparticles

Ceria nanoparticles loaded with edaravone have also been studied in relation to stroke. The particles have been modified with Angiopep-2 and poly ethylene glycol and proved to have an efficient BBB crossing through receptor mediated transcytosis. The particles were teste on healthy rats and proved to be able to cross the BBB and accumulate in the brain tissue. Moreover, they were able to preserve the integrity of the BBB. The

nanomaterial was also proved to be able to decrease infarct volumes in MCAO model animals [115].

Nanosystem	**Load**	**Result**
Glutathione coated PLGA-b-PEG	T3	Decreased tissue infarction and edema
PEG-PLGA-WGA	NR2B9	Improved ischemic damage
PLGA-CTX/LEX	NEP1-40	Reduced infarct size
Chitosan and glycerol tripalmitin coated sodium hyaluronate	-	Crossed BBB
PEGylated liposomes	-	Accumulation in ischemic core
PEGylated liposomes	Tacrolimus	Reduced apoptosis and neutrophil invasion Improved damaged area
T7&SHp-P-LP	ZL006	Crossed BBB, decreased neurological deficit
PEGylated liposomes	tPA	Increased tPA half-life
Chitosan coated PLGA	tPA	Accelerated thrombolysis
NE	Curcumin	Reduced hematoma size
Ceria	Edaravone	Decreased infarct size Crossed BBB

Table 4. *Reviewed nanosystems used to improve treatment of stroke, their load and the result of the study.*

7. Multiple sclerosis

Multiple sclerosis can be characterized as a chronic disorder of the central nervous system (CNS) that leads to demyelination, damage of the neurons and loss of axons [116]. The disease is most often seen in 20 to 40 year old people with women being twice as affected as men. The prevalence of the disease on a global scale is approximately 33 people per 100 000 but this is subject to a wide variability as we can see the highest prevalence figures in Europe and North America at over 100 people per 100 000. In contrast, Asia has a prevalence of only 2.2 per 100 000. Nonetheless, the figures continue to rise on a global level [117].

7.1 Pathogenesis of multiple sclerosis

Although the pathogenesis of multiple sclerosis is still unknown it is widely believed that an immunopathogenesis is involved. This notion is challenged by the fact it is based on an old artificial model that gave multiple sclerosis a superficial resemblance to experimental allergic encephalomyelitis. It implies that the disease is primarily a neurodegenerative disease caused by the disruption of the BBB. Clear risk factors have been identified though. These include smoking, UVB radiation as well as possible intake of highly unsaturated fatty acids [118]. Symptoms may arise from a monofocal or multifocal lesion in the CNS and can be monophasic, multiphasic (relapsing) or progressive in nature. There is a very wide variability in regarding the symptoms. Patients can complain of anything from blurred vision and weakness of the limbs to urinary incontinence and cognitive disorders. Nevertheless, multiple sclerosis remains difficult to treat [119].

7.2 Polymeric nanoparticles

A 2021 study explored the idea of delivering dimethyl fumarate with the use of chitosan-alginate nanoparticles through a biofilm oral formulation in order to improve bioavailability of the drug. The drug is a derivative of fumaric acid and is often used in treatment of multiple sclerosis. The biofilm was prepared and cut into 3.1cm by 2.1 cm rectangles. The study concluded that this administration represents a potential powerful alternative that could lead to increased bioavailability and a lower administered dose [120]. Chitosan (CS) has also been used in connection with sulfobutylether-β-

cyclodextrin (SBE-β-CD) loaded with IFN-β in IN administration. Chitosan has been of interest especially in the IN route administration due to its significant adhesion to mucosal surfaces. The study was carried out by using a preclinical EAE model (experimental autoimmune encephalomyelitis). The study also proved that INF-β in concentration of 25 000 IU and 50 000 IU had no major effect on cell viability. A concentration of 125 000 IU led to a decrease of cell viability of around 24%. It is noteworthy that only the nasal administration of the IFN-β NPs reduced damage in the experimental animals. The other groups, EAE untreated animals, free INF-β IN treated animals, systemically treated free INF-β animals and empty NP treated animals all suffered from degenerative processes. The authors concluded that this approach could represent a novel, non-invasive, cost-effective therapeutic alternative for multiple sclerosis [121].

7.3 Liposomes

A 2015 study used nano sterically stabilized liposomes (NSSL) loaded with methylprednisolone hemisuccinate and pegylated nano-liposomes loaded with Tempamine to treat EAE. Concerning the efficacy of the treatments, both of the tested formulations led to improvements over the saline control group. The study proved that treatment with 50mg/kg methylprednisolone hemisuccinate formulation achieved better therapy than free methylprednisolone hemisuccinate and current drugs like Betaferon and Copaxone [122].

7.4 Dendrosomes

Another study was carried out using biodegradable, amphipathic dendrosome NPs with the purpose of improving the solubility of curcumin. This study was also based on an EAE animal model of multiple sclerosis. The authors discovered that treating the experimental rats with the curcumin nanomaterial recovered the EAE rats and delayed the relapse of symptoms. Demyelination was also reduced in treated animals which points towards a promising multiple sclerosis treatment possibility [123].

7.5 DNA-peptide nanoparticle

A different approach was taken in 2014 by using a cationic nanoparticles made up of Tat 49-57 and MOG 33-35 (myelin oligodendrocyte glycoprotein) epitope and a plasmid

containing murine BLTA gene transduced in dendritic cells. BLTA is actually an inhibitor of the CD28 superfamily. This method was chosen due to the fact that multiple sclerosis is characterized by CD4+T cells that target myelin producing cells in the CNS. The nanoparticles were able to decrease both inflammation and demyelination [124].

Nanosystem	**Load**	**Result**
Chitosan alginate	Dimethyl fumarate	Increased bioavailability
SBE-β-CD	IFN-β	Reduced damage
NSSL	Methylprednisolone hemisuccinate	Improved therapy
Dendrosome	Curcumin	Reduced demyelination
DNA-peptide	Murine BLTA	Decreased inflammation and demyelination

Table 5. *Reviewed nanosystems used to improve treatment of multiple sclerosis, their load and the result of the study.*

8. Conclusion

Although nanomedicine technology is a relatively new approach to drug administration it is evident from the presented research that this method harbours tremendous potential. The nanoparticles mentioned in this literature review were able to elicit effects that reduced the pathogenesis or pathology of the mentioned neurodegenerative diseases.

The most notable results obtained by using nanoparticles in case of Alzheimer's disease were the reduction of Aβ plaque aggregation, increased BBB crossing and improved cognitive functions. As for Parkinson's most results pointed towards improved motor function, increased bioavailability and increased dopamine levels. In Huntington's disease lower oxidative stress levels and decreased HTT levels were observed among others. Studies concerning the use of NPs in case of stroke resulted in decreased infarct volumes, crossing the BBB, reducing hematoma size and increasing tPA half-life. Finally, for multiple sclerosis reduced demyelination and increased bioavailability may be mentioned.

It is also worth noting that a variety of administration routes were tested. For example, intravenous, intranasal, intraperitoneal and transcutaneous. Similarly, a wide range of NPs have been mentioned. By far, the most common used ones have been polymeric nanoparticles, namely, PLGA, PEG based nanoparticles and liposomes. Lipid based, gold and cerium particles have also been mentioned along others.

To sum up, the current review looked at the most recent research concerning the use of nanoparticles for drug delivery in relation to Alzheimer's disease, Parkinson's disease, Huntington's disease, stroke and multiple sclerosis. Overall, the literature suggest that NPs are a promising approach to drug delivery but require further testing and development. Nevertheless, they have the potential to revolutionize treatment of neurodegenerative diseases.

9. Summary

Due to the distinctive nature of the blood-brain barrier (BBB), advancement in neurodegenerative disease therapy is greatly limited. The continuous, non-fenestrated structure of the barrier prohibits the passage of all large molecules and approximately 98% of small molecules thus, inhibiting many therapy possibilities. Millions of people suffer from neurodegenerative diseases worldwide, but treatment is often inefficient or impossible. Nanoparticles represent a novel promising form of therapy; however, the technology is still in its primordial stages. Nonetheless, the benefits of nanosystems such as increasing drug bioavailability, increasing drug BBB crossing and drug delivery paired with their potential of drug dose reduction are revolutionary. The current review gives an overview of the most recent progress regarding nanomedicines as a means of drug delivery in the CNS for the purpose of aiding neurodegenerative diseases. Although many particle types can be found in the available literature, the most studied by far are polymeric coated or uncoated particles and lipid-based particles. Research around their implementation in the treatment of Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and stroke is abundant and many studies show that nanoparticles do improve these conditions in mouse models. Interestingly, a lot of effort is being put in developing intranasal administration nano formulations into case of Parkinson's disease. Overall, the literature points toward a favourable outcome with many of these formulations being successful at diminishing the effects and limiting the pathogenesis of the aforementioned neurodegenerative diseases.

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