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Role of Nanocarriers for Drug Delivery to Brain

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ABSTRACT

Noninvasive treatment of Neurological diseases is limited because of poor transport of drug molecules into brain. Almost all drugs and therapeutic agents do not reach to brain due to presence of defensive barrier of brain such as Blood Brain Barrier and Blood – Cerebrospinal Fluid Barrier. Therefore main challenge for CNS drug delivery is how to maintain therapeutic concentration of drug molecules in brain. Nanotechnology gives promising solutions to overcome these obstacles. Different nanocarriers such as Liposomes, Solid lipid nanoparticles, Micelles have been studies for the drug delivery across the brain. Many nanoformulations can transport drug molecules into brain in in vitro and in vivo models. Many studies have been done to access the nanotechnology against CNS disorders such as brain tumour, Alzheimer's disease and acute ischemic stroke. In future nanaocarriers will be promising alternative to increase brain drug concentration using novel drug delivery systems which will improve Blood brain barrier permeability and reduce their neurotoxicity.

Keywords: Nanocarriers; CNS; Brain drug delivery; Blood brain barrier

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ROLE OF NANOCARRIERS FOR DRUG DELIVERY TO BRAIN

Brain is most vital organ of body controlling all the actions. Blood Brain barrier is defense biological barrier which restricts the passage of toxins and pathogens but it also restricts the activity of drug molecule. For a drug to achieve its maximum activity it should cross blood brain barrier. To overcome these barrier different approaches has been made to increase brain drug concentration.

The CNS barriers protect the brain from invading pathogens, neurotoxin molecules and circulating blood cells. These barriers are Blood Cerebrospinal fluid barrier, Blood brain Barrier, Blood retinal barrier and blood spinal cord barrier [1]. Drug delivery to brain is limited due to presence of Blood Brain Barrier (BBB). Blood brain barrier restricts the passage of therapeutic agents, neuropeptides to brain. Many Drug Delivery methods have been developed for CNS diseases targeting but mostly are invasive and lack target specificity. To cross the blood brain barrier many types of nanocarriers systems has been made such as linear polymers, hyper branched polymers, dendimers, liposomes and micelles. Novel administrations has also been approached which includes temporary disruption of BBB to increase permeability, local drug administration by using impregnated polymers; Convection enhanced delivery and intranasal delivery. In this review brief introduction to BBB and role of nanocarriers for drug delivery to brain will be discussed.

Blood Brain Barrier

This is anatomic barrier of brain which is developed by coordinated function of multiple cell types to restrict passage of harmful substances to brain and maintains intra cranial pressure. BBB is formed by micro vascular endothelium, basement membrane and glial cells such as pericytes. Intranluminal space of brain capillaries are lined by monolayer of microvascular endothelial cells and closely packed endothelial cells forms the tight junctions limiting substances crossing the BBB [2]. Pinocytic vesicles and high number of mitochondria transport certain

molecules between blood and brain. BBB transport essential protein which are necessary for the brain function. Glucose transport (GLUT) system is essential for brain for Glucose gives proper energy and helps to function it properly. Modification of therapeutics molecules are done to be recognized by GLUT. Multiple Drug resistance Protein (MDR1) is another important transport system of Brain which restricts the transport of certain compounds in the brain [3]. To Cross the BBB, properties of barriers, permeability properties of brain endothelial cells and molecular weight of compound are important parameters. There are mainly four mechanisms of BBB transport [2].

Simple diffusion:
Transport through from high to low concentration.

Facilitated diffusion:
Type of carrier mediated endocytosis in which solute binds to specific membrane protein same like simple diffusion from high to low concentration.

Simple diffusion through an aqueous channel:
Formed within the membrane and mainly charged ions transport.

Active transport via protein carrier:
This is active transport occurred by a protein carrier having specific binding site that go through affinity changes. Concentration gradient is responsible for solute transport.
BBB is formed by cerebral endothelium, Blood – CSF barrier is comprised of choroid plexus and CSF-Blood barrier formed by avascular arachnoid epithelium which lies under dura and encloses the brain [4]. Anatomical and Physiologic functions of these barrier controls the toxicity and normal brain functions by regulating concentration and clearance of endogenous and exogenous molecules [5]. Brain endothelial has cells have tight junction complexes that restrict passage through BBB. Capillaries and endothelial cells occupy 1% and 0.1% of brain volume respectively, Surface area of brain microvasculature is ~20 m^2 and total length is ~400 miles. The distance between brain capillaries is ~40 µm and brain cells are at 20 µm from capillaries which forms spaces that allow small molecules to cross them [6]. But a feature of BBB does not allow molecules to pass through them. Hydrophilic compounds having mass lower than 150 Da and highly hydrophobic compounds having mass lower than 400-600 Da can cross the membrane by passive diffusion. Major Barriers of BBB are listed in Table 1 [7,8].

Table 1: Barriers of BBB

	Elements	Role
Anatomical Barrier	Tight interendothelial junctions	Restricting free exchange of solutes and cells between blood and CNS
	Absence of fenestrations and low number of pinocytotic vesicles	
	Luminal glycocalyx	
Transport Barrier	Diffusion pathways	Supply of sugars, amino acids, lipids, vitamins, minerals, methabolic precursors, peptides, protein
	Solute Carriers	
	Efflux Pumps	
	Adsorptive and receptor mediated trasnendothelial transport	
Metabolic barrier	Phase 1 and 2 enzymes	Protection from bioactive molecules

P- Glycoprotein is drug transport protein which is ATP – dependent located at apical membranes of different epithelial cells which forms BBB [9]. Presence p-gp can restricts the acitivity of many drugs in brain, including digoxin, dexamethasone, vincristine, taxanes, cyclosporine etc. [10]. For drug transport across the BBB primary physiological pathways which needs to be utilized are listed in Table 2 [8].

Table 2: Primary physiological pathways

Pathway	Transfer of	Methods to Enhance
Lipid – mediated diffusion	Lipophilic small molecules	Increasing lipophilicity
Carrier – mediated transport	Ligands of Carriers : amino acids, glucose and other nutrients	Development of ligand analogues
		Conjugation of drugs to ligand- targeted nanoparticles
Receptor – mediated transport	Peptides, proteins	Drugs or nanoparticles conjugates to peptide or protein vectors
Adsorptive – mediated transport	Serum Protein	Drugs or nanoparticles conjugated to cationic protein or peptide vectors

When neurological disease occurs, properties of BBB changes and these changes can be utilized for passage of drug molecules which are not possible in healthy brain. BBB functioning alters by oxidative stress, inflammatory mediators, lipid mediators, vasogenic agents, infective agents as well as physiological and immunological stimuli [11]. Table 3 contains the pathological conditions their action on BBB and how these changes can be utilized to transport the drug to brain [12].

Table 3: CNS disorders and their effect on BBB

Pathological conditions	Influence on BBB	Effects for Drug crossing BBB	References
Multiple Sclerosis	Disruption of Tj, Enhanced leukocyte activity, release of inflammatory cytokines/chemokines	It may enhance paracellular transport of drugs	[13-15]
Alzheimer's Disease	BBB disruption and allowed the greater access of peripheral IgG to CNS	Potentially it may enhance paracellular transport of drugs that have affinity for albumin and IgG into the CNS	[16]
	Overexpression of effluc pumps	Efflux pump inhibitors may improve drug deliver into the brain.	[17]
Parkinson's Disease	BBB disruption	It enhanced therapeutic agent concentration in the brain.	[18]
HIV	Increase in diameter of cortical vessels, thinning of basal lamina, loss of glycoproteins, apoptosis of endothelial cells and tight junction disruption	Potentially it may increase drug transport into the brain due to the leaky barrier.	[19]
Infectious disease	Leukocyte Invasion, elevated CSF - to – Serum albumin ratio and BBB impairment	It may enhance paracellular transport of drugs and drugs with affinity for albumin.	[20,21]
Inflammation	Increased BBB permeability	It may facilitate paracellular drug transportation.	[22]
Stroke	BBB disruption	It enhanced paracellular drug, e.g. Ginkgolide B, passage into the brain	[23]
	Upregulation of diphtheria toxin receptor	It may provide disease-induced specific drug targeting of the BBB and receptor mediated transcytosis.	[24,25]
Trauma	BBB breakdown	It enhanced therapeutic agent concentration in brain	[26]
Pain	Alternation of BBB chemokine receptor due to activated astricytes	It may lead to astrocyte-targeted therapy.	[27]
	Decreased Tight junctions proteins and BBB perturbation	It may facilitate paracellular drug transportation.	[28]
Brain Tumour	Loss of Tight Junctions in tumous vascular system, enhanced retention effect	Angiogenic vessels are permeable to nano-sized materials	[29]
	Overexpression receptors of folate, insulin and trasferrin	It enhanced folic acid, insulin and transferrin-attached nanoparticles across the BBB.	[30,31]
Ischemia /Seizures	Upregulation of Diptheria Toxin Receptors	Potentially it may increase disease-induced specific drug targeting of BBB and receptor mediated transcytosis.	[32]

To cross the BBB many methods has been developed which includes disruption of BBB, Carrier mediated transport and chemical modification of drug molecule. Intranasal route is being widely studied because of its potential to cross BBB through olfactory receptors. Nanocarriers are important drug delivery systems which can be utilized to cross the BBB and to deliver drugs to brain.

Approaches of Brain Targeting

Invasive approaches:

- Intaracerebroventricural infusion
- Convection – enhanced delivery
- Intra cerebral injection or implants
- Disruption of BBB

Noninvasive:

- **Chemical techniques**
  - Prodrugs
- **Colloidal techniques**
  - Nanoparticles
  - Liposomes
- **Miscellaneous techniques**

- Intranasal delivery

Invasive Approach
In this surgery is done by administering the drug in brain through implants or Intra cerebro ventricular infusion. These methods are complex and require neurosurgeons. Larger and smaller both molecules can be given through these methods.

ICV Injection
Drug distribution is limited to brain due to protective behavior of BBB, Intraerebroventricular drug administration is method that can cross BBB and high brain drug concentration can be achieved. In this method drug is directly administered into ventricles of brain. Factors which can affect drug efficacy are osmolarity, pH, preservatives used and diluents which are used in drug solution. Drugs can be administered intaventricularly using an Ommaya Reservoir; a plastic reservoir implanted subcutaneously is scalp and connected to the ventricles within brain vian an outlet catherer. Drug solution can be injected subcutaneously to implanted reservoir and delivered to ventricles by manual compression of reservoir through scalp [33]. This method is complex and required surgeons.

Limitations:
Due to presence of extracellular fluid space of brain drug diffusion is through brain parenchyma is very slow and inversely proportional to Molecular weight of drug [33].

Convection Enhanced Delivery
In this catheters of small diameter are directly placed in brain tumor. This reduces the surgical exposure of brain. Therapeutics is infused into tumor to saturate the target tissue. This allows macromolecular drugs to bypass the blood brain barrier. It minimizes the exposure of drug molecules to rest of body and drug can be delivered to targeted site. Convection enhanced delivery gives better drug distribution to brain tumors.

Limitations:
Back flow of drug with catheters and drug leakage in non desired areas.

Intracerebral Implants
In this technique therapeutic agents comprises of biodegradable polymer matrix or reservoir is implanted intracerebrally. This provides sustained drug release and localized delivery of drugs. Basic mechanism involves in this is diffusion and convection.

Limitations:
Drug distribution in brain decrease exponentially by distance to site of action required.

Blood Brain Barrier Disruption
By this medication pass through the protective blood brain barrier of brain and large doses can be send to tumor and nearby tissue. Blood Brain Barrier is made of tightly knit cells that line the blood vessels in brain. By shrinking these cells medication can be passed through brain and reach at targeted site. Some of the important techniques are:

Osmotic Disruptions
Endothelial cells shrinks by osmotic shock and disrupt tight junctions present in brain. Intracarotid infusion of a hypertonic arabinose or mannitol solution is used to cause vasodilatation and shrinkage of cerebrovascular endothelial cells. This method has been used to target water soluble drugs, peptides, antibodies and viral vectors for gene therapy. Metastatic or primary brain tumors are also treated with this method.

MRI Guided Targeted Blood Brain Barrier Disruption
This method is used to disrupt blood brain barrier noninvasively and reversibly at targeted locations. This technique is highly localized and can target wide range of therapeutic agents to brain. In 1942 Lynn et al. finds the potential of Focused ultrasound to produce thermal or mechanical effects in brain

Nanocarriers for Brain Drug Delivery
Nanocarriers are colloidal system of nano scale size able to transport drug molecules to different sites of body. They can carry small molecular weight drugs or macromolecules such as genes or proteins. Nanocarriers protect drug

from degradation, reduces the toxicity of molecules, increase solubility and bioavailibilty of drug molecules and can increase the half life in blood stream. Drug carrier can be made up of carbon, polymeric and magnetic materials. They can be utilized in targeted drug delivery and controlled drug delivery. An appropriate carrier is used in this formulation which is non-toxic, biodegradable, biocompatible and binds with drug without changing its chemical features.Nanocarriers such as liposomes and micelles can enhance the pharmacological properties of drugs because their small size (~100 nm or less) allows them to easily cross the biological membranes. In Table 4 list of nanocarriers which are nanopartiles and in Table 5 list of nanocarriers other than nanoparticels are listed respectively.

Table 4: List of nanocarriers used for CNS drug delivery

Carrier Type	Materials Used	Drugs	Results	Ref.
Polymeric nanoparticle	PBCA	Methotrexate	Increase Brian Drug Concentrartio	[34-36]
		Dalargin		
		Temozolomide		
	MMA-SPM, PCBA	Lamivudine(3TC)	Enhanced BBB permeability	[37]
		Zidovudine (AZT)		
	PLGA/Alginate	Dexamethasone	Extended drug release	[38]
	PLA/PEG	Vasoactive intestinal peptide	Increase Brain Drug concentration	[39]
	PLGA	Superoxide dismutase	Improved neurological functions	[40]
Solid lipid nanoparticle (SLN)	Soya phosphatidyl-choline 95%	Doxorubicin	Improved brain accumulation	[41,42]
	Stearic acid/ Soy bean lecithin	Camptothecin	Increases Drug Release	[43]
	Stearylamine	Paclitaxel	Enhanced Brain Drug Concentration	[44]
	Stearic Acid	Melatonin	Enhanced bioavalibilty	[44]
Lipid nanocapsule	Triglycerides of Capric and caprylic acids, Solutol	Etoposide	Improved Bioavalibilty	[45,46]
		Paclitaxel	Increased Half life	[44]
Albumin nanoparticle	Albumin	Loperamide	Improved BBB penetration	[47]
		Paclitaxel	Increase accumulation of Drug	[48]

Table 5: List of nanocarriers other than nanoparticles

Carrier type	Material	Drug	Result	Ref
Liposomes	Phospholipids and cholesterol	Phenytoin, γ-Aminobutyric acid	Improved local action	[49]
		Cisplatin	drug concentration	[50]
		Stavudine	Enhanced Anti HIV Increased effect	[50]
		Amphotericin	Increased Brain Drug concentration	[51]
Micelles	Pluronic P85	Biphalin, Enkephalin, Morphine	Enhanced analgesic properties	[51,52]
	Core Shell Micelles	Doxorubicin, Digoxin, Paclitaxel, Ritonavir, Vinblastine,	Increased brain drug concentration	[52-54]
		Antioxidant nitroxyl radicals	Improved Neurological functions	[55]
Dendrimers	Mannosylated Poly(propyl eneimine)	Lamivudine	Increased uptake of Lamivudine	[56]
	Polyether-copolyester	Methotrexate	Enhanced circulation	[57]
Nanogel	N-isopropyl-acrylamide\N-vinyl-pyrrolidone	5-fluorouracil	Increased Brain accumulation	[58]
Nano-Emulsion	Edible Oil	Saquinavir	Increased Brain drug Concentration	[59]
	Pine nut oil	Paclitaxel,Ceramide	Increased Drug Uptake	[60]
Nano suspension	Atovaquone Crystal	Atovaquone	Enhanced Drug bioavailability	[61]

Liposomes

These are nano or microsize vesicles which consist of one or more lipid bilayers surrounding an aqueous compartment. These are spherical shape vesicles composed of cholesterol and phospholipids they have ability to incorporate hydrophilic, lipophilic and hydrophobic compounds. Hydrophilic compounds can be entrapped into aqueous core of liposomes or between the lipid layer and water phase. Lipophilic or hydrophobic compounds are

incorporated into hydrophobic core of lipid bilayers. With the incorporation of polymers, polysaccharides, peptides, antibodies their surface can be modified to improve brain drug delivery. Table 6 comprises of advantages and disadvantages of liposomes system. In Tables 7 and 8 applications of liposomes and marketed prerparations of liposomes are listed respectively.

Table 6: Advantages and disadvantes of liposomes [62]

Advantages	Disadvantages
Liposomes are increased efficacy and therapeutic index of drug (Actinomycin-D).	Production cost is high.
Liposome is increased stability via encapsulation.	Leakage and fusion of encapsulated drug / molecules.
Liposomes are biocompatible, completely biodegradable, non-toxic, flexible and nonimmunogenic for systemic and non-systemic administrations.	Sometimes phospholipid undergoes oxidation and hydrolysis like reaction
Liposomes are reduction in toxicity of the encapsulated agent (Amphotericin B, Taxol).	Short half-life.
Liposomes help to reduce exposure of sensitive tissues to toxic drugs	Low solubility
Site avoidance effect.	Fewer stables
Flexibility to couple with site-specific ligands to achieve active targeting	

Liposomes Mechanisms of Crossing BBB

Cationization of vector:

At blood brain barrier electrostatic interaction is present between positive charge and polyanious which can lead to adsorptive mediated endocytosis [63,64].

Tageting ligand:

Receptor mediated transcytosis can occur by using ligand targeting liposomes toward the receptors present on brain endothelial cells. Antibodies and aptamers can bind to liposome surface [65,66].

Triggered drug release:

Magnetic field, temperature, ultrasound intensity, light or electric pulses are specific external stimuli which can trigger drug release of liposomes [67,68].

Theranostic:

Non invasive contrast agents can also entrapped in lipsomes. For the diagnosis, real times monitoring of disease multifunctional theranostic liposomes are used [68,69].

Table 7: Applications of lipsomes [70]

Actions	Examples
Improved solubility of lipophilic and amphiphilic drugs	Amphotericin B, porphyrins, minoxidil, some peptides, and anthracyclines, respectively; hydrophilic drugs, such as anticancer agent doxorubicin or acyclovir
Passive targeting to the cells of the immune system, especially cells of the mononuclear phagocytic system	Antimonials, amphotericin B, porphyrins, vaccines, immunomodulators
Sustained release system of systemically or locally administered liposomes	Doxorubicin, cytosine arabinoside, cortisones, biological proteins or peptides such as vasopressin
Site-avoidance mechanism	Doxorubicin andamphotericin B
Site-specific targeting	Anti-inflammatory drugs, anti-cancer, anti-infection
Improved transfer of hydrophilic, charged molecules	Antibiotics, chelators, plasmids, and genes
Improved penetration into tissues	Corticosteroids, anesthetics, and insulin

Table 8: Marketed liposomes products [71]

Product	Drug	Formulation	Company	Indication/Target	Country
Doxil ™	Doxorubicin	Liposomes (LCL)	Sequus Pharmaceuticals,Inc., CA	Kaposi sarcoma in AIDS	USA and Europe
Ambisome ™	Amphotericin B	Liposomes (CL)	NeXstar Pharmaceutical Inc., CO	Serious Fungal Infections	In 24 countries
DaunoX-ome ™	Daunorubicin citrate	Liposomes (LCL)	NeXstar Pharamceutical Inc., CO	Kaposi sarcoma in AIDS	USA and Europe
Amphocil ™	Amphotericin B	Lipid Complex	Sequus Pharamceutical Inc., CA	Serious Fungal Infections	Asia and Europe
Abelcet ™	Amphotericin B	Lipid Complex	The Liposome Company, NJ	Serious fungal Infections	USA and Europe

Solid Lipid Nanoparticles

In 1991 solid lipid nanoparticles were introduced which gives alternative carrier system to traditional colloidal carriers, such as emulsions, liposomes and polymeric micro and nanoparticles. They have many advantages over the traditional drug delivery system but lesser disadvantages as compared to them [72]. Poly(ethylene glycol)- modified SLNs can penetrate BBB and allow great delivery to brain [73]. For the manufacturing of SLN Lipids are used,which are solid at room temperature and also at room temperature, such as triglycerides, Partial glycerides, fatty acids, steroids and waxes.

Advantages of SLNs [74]

- Possibility of controlled drug release and drug targeting
- Increased drug stability
- High drug payload
- Incorporation of lipophilic and hydrophilic Drugs
- No biotoxicity of carrier
- Avoidance of organic solvents
- No problems with respect to large scale production and sterilization

Disadvantages of SLN

- Poor Drug loading
- Drug Explusion After polymeric transition during storage
- Relatively high water content of dispersions (70-99.9%)

Advantages of SLNs Over Polymeric Nanoparticles

Reticulo Endothelial System cannot take up SLNs ranging 120-200 nm and bypass liver and spleen filtration [75]. For weeks controlled can be achieved and site specific delivery can be possible by attaching ligand and by coating [76,77]. SLNs are very stable as compared to other formulations and have high drug payload [43]. They can entrap both hydrophilic and hydrophobic drugs [73,78]. Mostly carrier lipids are biodegradable and safe [79] and less usage of organic solvent [80].

Microspheres

Microspheres are also referred as microparticles. These are small spherical particles ranging from 1 μm to 1000 μm. These are free flowing powder consisting of proteins, starches, gums, fats and waxes and synthetic biodegradable polymers. Albumin and Gelatin are natural polymers and Poly lactic acid and polyglycolic acid is synthetic polymer which is used in microspheres. Polymer microspheres comprised of polyethylene and polystyrene are most commonly used. Polystyrene microspheres are used in biomedical applications and polyehtlene microspheres as temporary and permanent filler. Glass microspheres have limited applications in medical field. Ceramic microsphers are used as grinding media. In Table 9 Applications of microspheres for CNS delivery are listed

Materials Used in Preparation of Microspheres [81,82]

Classification:

- Synthetic polymers
- Natural Polymers

Synthetic polymers:

Non bioderadabale polymers: Polymethyl methacrylate (PMMA), Acrolein, Glycidyl methacrylate, Epoxy Polymers.

Biodegradable Polymers: Lactides, their glycolides and their copolymers, Polyalkyl Cyano Acrylate, Polyanhydrides.

Natural Polymers:

Obtained from Sources like proteins, carbohydrates and chemically modified carbohydrates.

Proteins: Albumin, Gelatin, Collagen.

Carbohydrates: Agarose, Carrageenan, Chitosan, Starch, Chemically modified Carbohydrates like Poly (Acryl) Dextran, Poly (acryl) Starch.

Advantages

They provided sustained release of drug and minimize dosing frequency and improve patient compliance. Due to Smaller size and spherical shape can to given intravenously. They enhance the bioavailability and minimize adverse effects of drug.

Limitations

Incorporation of food and rate transit though gut can alter the release rate of controlled release dosage form. Release rate may be differing from one dose to another in prolonged release. Sustained and controlled release formulations usually contains higher dose of drug which can lead to toxicity.

Applications

Bioadhesive microspheres:

- Biodegradable Starch microspheres for Nasal delivery of Domperidone [83].
- Nasal delivery of insulin by degradable microspheres [84].
- Chitosan – ethylcellulose Microspheres for nasal delivery [85].
- Mucoadhesive microspheres for nasal administration of metoclopramide[86].
- Mucoadhesive microspheres for gastrointestinal tract [86].
- Microspheres for sustained ocular delivery of daunorubicin [87].
- Bioadhesive sulfacetamide sodium microspheres for treatment of bacterial keratitis [88].

Magnetic microspheres:

- Thermo- sensitive magnetic hygrogel microspheres for enzyme immobilization [89].
- Low dose of doxorubicin loaded magnetic albumin microspheres for selective targerting [90].
- Magnetic microspheres for labeling and separation of cells [91].

Floating microspheres:

- Gastroretentive floating drug delivery of repagalinide by calcium silicate based microspheres [92].
- Prolongation of gastric residence time of Cimetidine by floating microspheres [93].
- Floating microspheres of metformin hydrochloride for controlled release [94].
- Floating bioadhesive microspheres of acetohydroxamic acid for clearance of helicobacter pylori [95].

Radioactive microspheres:

- Radioactive microspheres to assess distribution of cardiac output in rabbits [96].
- Targeted delivery of magnetic radioactive 90 Y-microspheres to tumor cells [97].

Table 9: Application of microsphers for neurodegenrative diseases

Polymer	Microsphere size	Drug	References
Hyaluronane derivative	2-21 µm	Nerve growth factor	[98]
Alginate-polylysine	50 µm	Nerver growth factor	[99]
Alginate-Chitosan	50 µm - 3 µm	BDNF	[100]
Poly(DL-lactide-co-glycolide)	5-45 µm	Dopamine	[101]
Poly(DL-lactide-co-glycolide)	17.2 µm	Nerve growth factor	[102]
Poly(DL-lactide-co-glycolide)	2.5 µm	Monosialoganglisoside + Nerve Growth Factor	[103]
Poly(DL-lactide-co-glycolide)	2.6 µm	Brain derived Neurotrophic Factor	[100]
Poly(DL-lactide-co-glycolide)	8-11 µm	Nerve Growth Factor	[104]
Poly(DL-lactide-co-glycolide)	50 µm	Nerve Growth Factor	[104]
Poly(DL-lactide-co-glycolide)	25 µm	Nerve Growth Factor	[103]
Poly(DL-lactide-co-glycolide)	1.8 µm	Ciliary Neurotrophic Factor	[103]
Poly(DL-lactide-co-glycolide)	2 µm	Ciliary Neurotrophic Factor	[105]

Dendrimers

These are class of polymeric materials that are nano sized symmetrical structure consisting of tree like atoms. The word “dendrimer” originated from two words, Greek word “Dendron” meaning tree, and “meros” which mean part. Dendrimers are monodisperse marcomolecules that contain symmetric branching units built around a small molecule or linear polymer core. Dendrimers structure consists of three components:

- A initator

- interios layers or repeating units
- Exterior layer with outer interior generatios

Dendrimers of different compositions are explored for drug delivery which includes Poly(aminoamine (PANAM), Poly-(Etherhydroxylamine) (PEHAM) and Poly (propyleneimine) (PPI) dendrimers [106]. PANAM have uniqure strutersa and properties. Polycationic dendrimer having primaty amine on surface are Full generation PANAM and Polyanionic dendrimer that has carboxylic acids on surface are half generation [107]. Polyanionic dendrimers are less toxic than polycationic dendrimers [107]. PANAM dendrimers for CNS delivery can be synthesized [108-110]. Dendrimers are modified with spacers for bioavailability improvement, buffer capacity and half-life. Surface modified dendrimers are conjugated with specific ligands to target BBB. Surface modified dendrimers complexed with gene therapeutics or drug. Imaging agents covalently conjugated with dendrimer used for *in vivo* imaging and diagnosis. Tables 10 and 11 consist of Dendrimers generations and therapeutics applications of dendrimers. In Table 12 list of PANAM dendrimers for CNS delivery is listed.

Table 10: Dendrimers generations

Dendrimers Generation	PANAM (No of Branches)	PPI (No of Branches)	Phosphorus Dendrimers
0	4		
1	8		
2	16	8(16)	24
3	32	16(32)	48
4	64	32(64)	96
5	128	64(128)	192

Table 11: Therapeutic applications of dendrimers

Application	Dendrimers	Drug	Reference
Solubilization	PPI Dendrimers	Amphotericin B Famotidine	[111]
Enhanced cellular uptake	Mannosylated PPI dendrimers	Efavirenz	[112]
Biocompatible Drug carrier	Mannosylated PPI dendrimers	Rifampicin	[113]
Drug Targeting	Mannosylated PPI dendrimer		[113]
	Folate conjugated dendrimers		[114]
	Dextran conjugated PPI dendrimers		[115]
Sustained release	Dextran conjugated PPI dendrimers	Doxorubicin HCl	[115]
Delivery of anti-HIV drug	4.0 G PPI	Zidovudine	[116]
Delivery of anticancer bioactives	4.0 G PAMAM	Doxorubicin	[117]

Table 12: List of PANAM dendrimers for CNS delivery

Generation	Linkage	Drugs delivered	Ligand(s)	Major findings and comments	Ref
G4	MAL-PEG5000-NHS	Doxorubicin	Transferrin (Tf) and wheat germ agglutinin (WGA)	Reduced nonspecific uptake by the normal cells; enhanced transport across the BBB	[118]
	MAL-PEG5000-NHS	Doxorubicin	Transferrin (Tf) and tamosifen (TAM)	Enhanced transport across the BBB	[119]
	NHS-PEG3400-MAL	pEGFP-N2 plasmid	Lactoferrin (Lf)	Enhanced brain uptake and transfection e fficiency	[120]
	Triglycine (GGG)	Quantum dots (Qdots) and YFP siRNA plasmid	epidermal growth factor (EGF)	Enhanced nucleic acid delivery compared to trans-IT	[121]
G5	NHS-PEG3400-MAL	pEGFP-N2 plasmid	rabies virus glycoprotein (RVG29	Enhanced *in vivo* biodistribution in the brain	[109]
	NHS-PEG3400-MAL	pEGFP-N2 plasmid	Transferrin (Tf)	Enhanced brain uptake and brain transfection e fficiency	[110]
	NHS-PEG3400-MAL	pEGFP-N2 plasmid	Angiopep-2	Enhanced brain uptake and transfection e fficiency	[108]
	NHS-PEG3400-MAL	pORF-TRAIL plasmid	Angiopep-2	Enhanced *in vivo* biodistribution in the brain	[122]
	NHS-PEG3400-MAL	pORF-TRAIL plasmid	Chlorotoxin (CTX)	Enhanced *in vivo* biodistribution in the brain	[123]
G4.5	NHS-PEG3400-MAL	opioid peptide DPDPE	Transferrin receptor monoclonal antibody OX26	Enhanced permeability through buccal mucosa by multiple fold	[124]

Micelles

Micelles are amphilic molecules aggregate present in aqeous medium [125] they can entrap poor water soluble, lipophilic compound in their micelles core. Mostly studies micelles are Pluronic based micelles. They can deliver the drug to Brain by crossing the blood brain barrier [54,125]. Micelles composed of Pluroric P85 Shows Enhancement of upto 19 fold in bovine brain microvessel endothelial cell line [52,54]. Polymeric micelles enhanced the delivery of Ritonavir, vinblastine and paclitaxel by suppressing the MDR1 mediated Drug efflux [54,52]

Micelles does not increase brian drug level in MDR 1a/b mice because of ATP depletion [126] Table 13 consists of studies done using Micelles for solubility enhancement.

Table 13: Solubility enhancement using micelles

Drug	Amphiphilic polymer	Comment	Ref
Camptothecin	Pluronic P105, d- α -tocopheryl polyethylene glycol 1000 succinate	Increased micellar stability; increased cytotoxicity	[127]
Docetaxel	Poly(ethylene oxide)-blockpoly(styrene oxide) (PEO-b- PSO) and PEO-b-poly(butylene oxide) (PEO-b-PBO)	PSO-based copolymers were associated with higher solubilizing capacities than PBO due to the aromatic structure of the coreforming polymer	[127]
Griseofulvin	E B copolymers (E = oxyethylene, m n B = oxybutylene, subscripts denote number-average block lengths in repeat units)	Solubilization independent of B block length when it exceeds about 15B units	[128]
Paclitaxel	N-octyl-O-sulfate chitosan	Improved bioavailability and reduced toxicity	[129]
Paclitaxel	mixed micelles of polyethylene glycol-phosphatidyl ethanolamine (PEG-PE) and vitamin E	Mixed micelles efficiently solubilized poorly soluble drug as compared to PEGPE micelles	[130]

Nose to Brain Drug Delivery

Through the olfactory or trigeminal nerve system that ends at olfactory neruoepithelium or respiratory epithelium drug delivery to brain can be possible through nasal route [131]. BBB can be crossed by using these nerve systems. Nose to brain drug delivery can reduce systemic toxicity but this route is inefficient. According to Illum et al. less than 0.1% of drug through nasal route will normally reaches to brain [132]. Poor nose to brain drug delivery can be improved using nanocarriers. Main barrier of this route is olfactory epithelium. Drug loaded nanocarriers can be used to achieve transmembrane transport across the barrier. Many studies have been done which suggests this strategy. A few studies have provided supportive data to this strategy. According to Betbeder et al. analgesic effect of morphine can be increased by administering morphine nasaly using 60 mm maltodextrin nanoparticles [133]. This study also concludes that Intransal nanoparticles formulation shows superior analgesic effect against subcutaneously administered morphine. Zhang et al. done same study using intranasally administered nanoparticles of nimodipine which shows improved results using nasal route [134]. Mucoadhesive nanopaticles can improve the olfactory drug delivery. Mainly Chitosan is used as mucoadhesive agent that can interact with junctional compleses between epithelial celles. Estradiol and risperidone were given intranasaly using Chitosan nanocarriers and their therapeutic effects were stronger than intravenously administered nanocarriers [135,136]. Lectins can also be used as mucoadhesive [137]. Lectin coated PLA nanoparticles can increase the coumarin concentration in brain by two folds instead of uncoated ones.

Neurotoxicity of Nanocarriers

CNS in highly protective system but nanocarriers can cross them which can also lead to over exposure of drug molecules and nanomaterials resulting in CNS toxicity. *In vitro* and *in vivo* neurotoxicity is reported in many studies using nanocarriers. In table shows these studies [48,138-141]. For *in vitro* neurotoxicity evaluation PC12 neuronal cell line is commonly used [138,142]. Brain damage is generally causes by increasing Reactive oxygen species levels and they can further exploited using neural stem cells in *in vitro* studies and *in vivo* studies [48,140]. Titanium oxide nanoparticles does not directly cause damage to dopaminergic neuronal cells, butt can cause damage to microglial cells [48]. Table 14 consists of several studies showing neurotoxicity caused by nanaocarriers. Mostly neurotoxicity studies performed using nanoparticles which are made of inorganic material. In future, toxicity of polymeric and lipid nanocarriers loaded drug should be conducted to find out potential risks along with targeted drug delivery.

Table 14: Neurotoxicity of nanocarriers for CNS delivery

Tested model	Carrier Type	Major Findings	References
PC12 Neuronal cell Line	Manganese oxide nanoparticles	Increased levels of Reactive oxygen species	[143]
	Copper, silver, manganese nanoparticles	Toxicity of Dopaminergic	[144]
	Anionic magnetic nanoparticles	Decrease in cell viability and altered nerve growth factor	[145]
Mouse Neural stem cells	Zinc oxide nanoparticles	Cell apoptosis	[146]
Dopaminergic neuronal cells	Titanium oxide nanoparticles	Neuronal cell toxicity	[138]
Mice model	Ultrafine carbon black particles	Changes inflammatory cytokines	[139]
Rat model	Manganese oxide nanoparticles	Increase in macrophage inflammatory protein-2	[142]
Fish model	Fullerenes nanoparticles	Lipid peroxidation, protein damage and glutathaione depletion	[142]

CONCLUSION

Nanotechnology is suitable for targeted drug delivery and improves the therapeutic management of Brain diseases. Pharmaceutical industry in moving toward the nanotechnology because the can cross the BBB and improve patient health. But many issues have been reported using nanotechnology for Brain diseases. Because of complex structure of brain and toxicity produce by nanomaterials, toxicity studies are required. Nanocarrier medicine should be evaluated for both with and without drug molecules. Polymers or other matierals which has been used should in therapeutic range and should not cause any toxicity and interfere with drug molecule action. Mostly Brain diseases require emergency treatment so chronic and cumulative effect of nanoformulation should be checked on brain tissues and toxicity studies should also be done. Brain is very vital organ damages to this organ are very difficult to evaluate as compared to other organs such as liver, heart or kidney. Many advanced diagnostic techniques are employed to diagnose brain diseases such as Magnetic resonance imaging, positon emission tomography and computed tomography scan. Targeting drug delivery is important science to cure the chronic diseases. Many targets like LDL receptors, Insulin receptors and transferring are important in targeted drug delivery. CNS targeting can caused by carrier mediated transporters and endogenous carriers also be exploited for brain targeting.

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