Chapter 2
Nanocarriers as CNS Drug Delivery Systems for Enhanced Neuroprotection

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Abstract Blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BSCFB) separate central nervous system (CNS) from blood circulation and tightly protect CNS by hindering the passage of harmful substances across the barriers and allowing the transport of essential compounds for brain function. These selectively permeable barriers have become a major challenge in delivering drugs into the nervous system for the treatment of CNS diseases and complications such as Alzheimer's disease, Multiple sclerosis, and encephalitis. Due to the advancements in nanotechnology and medicine, nanocarrier-based drug delivery has emerged as a new and potential strategy for neurological treatment and protection. Depending on the techniques in preparation and functionalization, nanocarriers may be developed with different properties for cell-/tissue-/organ-specific targeted delivery and for sustained and controlled release of neurotherapeutic agents. Even though several attempts have shown successful results in effective drug delivery to the central nervous system, the lack of information about long-term nanocarrier toxicity, accumulation, and excretion restrict their use in current clinical practice. This chapter

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highlights recent developments in nanocarriers specifically designed to protect CNS. The interactions between nanocarriers including liposomes, micelles, organic and inorganic nanoparticles, nanofibers, and carbon-based materials with various neuroprotective agents, and their capabilities of delivering the encapsulated or conjugated drugs to the CNS are reviewed. The review also includes our investigations on the development of titanate nanospheres and nanowires and their potential use as drug delivery tools in neuroprotection. Finally, future prospects of drug delivery systems in the treatment of neurodegenerative pathologies for clinical translation are described.

Keywords Drug delivery • Nanocarriers • Nanowires • Neuroprotection • CNS diseases • Blood-brain barrier

1 Introduction

Central nervous system (CNS) is a very complex structure that is well separated from the fluctuating blood surroundings with specialized barriers, namely blood brain barrier (BBB) and blood-cerebrospinal fluid barrier (BSCFB) [1, 2]. Previous research has shown that in the brain structure, there is a substantial correlation between the capillary endothelial cells and the blood-brain barrier [1]. Water soluble molecule's paracellular diffusion is hindered by BBB due to the sophisticated endothelial cell matrix. Blood–cerebrospinal fluid barrier also exhibits comparable barrier properties to those reported for BBB as a result of complex choroid plexus epithelial cells [1, 3]. Alongside its unique barrier behavior, it also facilitate ions and nutrients to diffuse into the cerebrospinal fluid (CSF) while it discharges toxic substances from the cerebrospinal fluid by employing the various transport process [3].

A key limitation of delivering drugs to the CNS is that there are very few methods available to pass through the abovementioned barriers. For several years, great effort has been devoted to the study of developing effective strategies to penetrate the barriers for the treatment of CNS diseases and disorders such as Alzheimer's disease, Parkinson's disease, Multiple sclerosis, autism, and encephalitis. The most common strategies can be categorized as; modification on drugs, manipulating the BBB, chemical drug delivery, and carrier and receptor mediated drug delivery approaches [2, 4]. The outcome of most of the drug modification research have been unsuccessful. Their major drawbacks includes high toxicity, low efficiency, detrimental protocols and safety hazards [4].

Researches have been continuing to find unorthodox solutions for effectively delivering drugs. Nanocarriers have appeared as promising approach in delivering drugs to the aimed target within the central nervous system and effectively traversing the BBB and BSCFB. It is well known that the size, surface charge, and morphology of nanocarriers have huge impacts on their passage through CNS barriers [5–7]. Even though the detailed mechanisms of this are still unclear, the increased

therapeutic effect by nanoparticulate delivery may be attributed to various possible mechanisms: (1) nanoparticle adsorption to the blood capillary walls results in prolong nanoparticle-barrier interaction, which allows drug to pass through BBB at high concentration level [2], (2) nanoparticles disturb tight junctions and form gaps for drugs to cross through the BBB [3], (3) endothelial cells uptake the nanoparticles through endocytosis and transfer drugs into the brain, and (4) endothelial cells deliver the nanoparticulate system through transcytosis [3].

In this section we review recent findings of delivering nanocarriers such as liposomes, micelles, organic and inorganic nanoparticles, nanofibers, and nanotubes into the CNS, and common drug loading methods. Additionally, our titanate nanofiber synthesis method and its use as nano-drug delivery system is discussed.

2 Nanoporous Liposomes and Micelles

Liposomes are self-assembled small spherical vesicles composed of one or multiple layers of phospholipids/lipids with a hydrophilic compartment at the center [8]. Due to the non-covalent interactions such as Van der Waals forces and hydrogen bonding between molecules, liposomes are reversible structures [9]. They have been extensively investigated since 1960s, and they have known as the ideal drug carriers to date since they have had the greatest impact in both clinics and market [10].

Morphological resemblance of a cell membrane and a liposome makes these lipid vesicles a strategic approach in the use of drug delivery systems. Liposomes are biodegradable, and their surfaces can be functionalized with ligands to prolong the circulation time and target to specific cells, tissues, and/or organs [10]. Therefore, the primary focus in research is on the surface decoration of liposomes for their clinical translation. However, optimization of liposomes for their size, encapsulation efficiency, and circulation time, sterilization, and cost-effective scale up technology are significant parameters that need to be considered [11].

Polyethylene glycol (PEG) coating on liposomes is a successful advancement for avoiding from reticuloendothelial system (RES) recognition [8]. Further research on PEGlyated liposomes have continued to improve their stability, uptake from specific cells and penetration through barriers with glutathione-modification [12, 13]. Modification of liposomes with cell penetrating peptides (CPPs) is another approach to enhance their efficacy in crossing the barriers and improve cellular uptake to achieve therapeutic success [14].

Liposomes have been also modified for brain tumor treatment [15–17]. For example, specific ligand transferrin (T7) and non-specific cell-penetrating peptide (TAT) were linked on the surface of doxorubicin encapsulated liposomes for cell targeting specific to brain glioma and access through BBB [18]. In a similar study, CPPs with different hydrophilicity, hydrophobicity, and amphiphilicity, namely TAT, Mastoparan, and Penetratin respectively, were attached on drug loaded liposomes and compared for their performance in penetration and localization [14]. Other than tumors, infections may also cause neurological complications. For

instance, a water-soluble steroidal prodrug, β-methasone hemisuccinate (BMS), was encapsulated by liposomes and investigated for their effect in inflammatory response in-vivo for cerebral malaria treatment [19].

Liposomes when used as carriers for drugs, their administration is mostly intravenous. However, oral and nasal administration routes are being considered now for the advantage of non-invasive techniques. In a study, nipodimine proliposomes, which form liposomal structure upon contacting with water, were prepared to increase the oral bioavailability of the drug [20]. In another study, specific compounds such as *α*-tocopherol (Toc) and omega3 fatty acid were encapsulated into liposomes and co-delivered with anti-Alzheimer drug tacrine (THA) into the neuronal-like cells for the treatment of Alzheimer's disease with intranasal route [21].

Micelles are vesicles that are composed of amphiphilic surfactants (non-polymeric micelles) or amphiphilic copolymers (polymeric micelles) [22]. In both cases, the outer site of the membrane is hydrophilic and therefore the micelle becomes water soluble. In contrast, the inner core of the micelle is hydrophobic, which allows hydrophobic drugs to be transported [23].

Polymeric micelles have been attracted due to better stability than non-polymeric micelles. Additionally, they can be functionalized specific to target and can be triggered with external stimulants (ultrasound, temperature, pH, etc.). Studies demonstrated that polymeric micelles loaded with anti-tumor agents showed enhanced drug transport across the BBB for the treatment of brain tumors [24–26].

Drugs can be loaded into liposomes either during liposome formation (i.e., passive loading) or after vesicle formation (i.e., active loading). Mechanical dispersion (e.g. sonication, micro-emulsification, membrane extrusion, lipid film hydration, etc.), solvent dispersion (e.g. reverse phase evaporation, ethanol injection, and ether injection), and detergent removal methods (e.g. dialysis, gel-permeation chromatography etc.) are examples of passive drug loading [27]. In these cases, drug is mixed with the solution during liposome manufacturing process. On the other hand, ionizable drugs are introduced to the liposomes based on the pH difference between inside and outside of the liposome membrane [28].

3 Hollow and Porous Nanostructures

3.1 Motivations

Current treatments for neurodegenerative diseases and injuries mostly fail because enzymatic degradation and systematic clearance of therapeutic agents in biological systems cause them to reach to the defect site at low-concentration. Agents at the site of injury further come across with the Central nervous system (CNS) barriers, namely blood brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSFB), limit their access to CNS resulting in poor treatment efficacy [1, 4, 29]. Therefore, delivering neuroprotective agents to the CNS has been a great challenge for decades [30].

Nanoparticles have been found to be a promising approach to overcome these limitations [6]. Nanoparticles are nanometer-size colloidal systems ranging from 1 to 1000 nm [31]. They can be made of various materials (e.g. polymers, metals, ceramics, etc.) and in different shapes (e.g. cubic, spherical, rod-like etc.) depending on the applications [32–34].

3.2 Successes in Porous Polymeric Nanostructures

Polymers are probably the most intensively studied material because of their biocompatible, biodegradable, and tunable structural properties [23]. Polymeric nanoparticles may be prepared from synthetic polymers such as poly(lactic-co-glycolic acid) (PLGA) [35, 36], poly(ethylenimine) (PEI) [37], poly(lactic acid) (PLA) [38], and poly(ε-caprolactone) (PCL) [39, 40], or natural polymers such as chitosan [41, 42], gelatin [43, 44], dextrane [45], and alginate [46, 47]. Among them, being an FDA-approved copolymer makes PLGA a widely studied material as a CNS delivery vehicle [35]. Tissue inhibitor of matrix metalloproteinases 1 (TIMP-1) loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles—with and without coating with polysorbate 80 (Ps80)—were tested in-vitro and in-vivo for their enhanced endothelial cell barrier permeability [48]. The results indicated that binding to the rat brain capillary endothelial cells (RBCEC) increased in uncoated nanoparticles while the penetration enhanced in Ps80 coated nanoparticles [48]. In another study, PLGA-huridin/Pluronic F-127 injectable system was developed to reduce the burst release effect and sustain the release of the thrombin inhibitor [35]. In this study, double emulsion solvent evaporation technique was used to encapsulate huridin into the PLGA microspheres, and they were further incorporated into F-127 hydrogel prior to local injection into mice. Although 50% of the loaded drug was released from the carrier within 8 h, 48 h sustained release was achieved in-vitro. Besides, in-vivo studies demonstrated reduction in the population of macrophages that are associated with gliosis, increase in the population of oligodendrocyte progenitor cells, which are responsible for remyelination, and increase in the recovery of motor function of animals [35]. In another study, receptors, namely Rabies virus glycoprotein (RVG), a 12 amino acid-peptide (TGN), and adenosine (Ad), which plays important roles during receptor-mediated trans cytosis, were linked onto the polymeric nanoparticles (i.e. poly(ethylene glycol) (PEG), coated poly(lactic-co-glycolic acid) (PLGA) and hyperbranched polyglycerol (HPG)–poly(lactic acid) (PLA) NPs) and tested for their accumulation in the mice brain after injected intravenously [49]. Furthermore, camptothecin (CPT) was loaded into the PLA-HPG-Ad NPs, and effective delivery of the agent into the intracranial gliomas was achieved [49].

Besides synthetic polymers, natural polymers have been intensively investigated as neuroprotection agent carriers [32]. Zhu et al. performed a comparative study to investigate if nanomaterial-nerve growth factor (NGF) interaction has better effect over the growth factor alone on recovery in spinal cord injury [50]. For this purpose,

NGF was loaded into the gelatin nanostructured lipid carriers (GNLs) via water-in-water emulsion method. The rats were intravenously treated with the same concentration of NGF with and without GNL. The results showed that Basso, Beattie, and Bresnahan locomotion and inclined plane test scores were significantly higher in NGF-GNL than NGF group after 14 days of injection. Superior neuroprotective effect of therapeutic agent loaded GNLs were further proved by Hematoxylin–Eosin staining of spinal cords [50]. Furthermore, immunofluorescence staining revealed that the target of NGF-GNL was ER stress-induced apoptosis proteins [50].

Proven as having antioxidative and anti-inflammatory properties, chitosan has become a potential candidate for neuroprotection material [51–53]. Chitosan nanoparticles (100–200 nm in size) that were tested in a guinea pig model inhibited the enzyme leakage from the damaged cells by sealing them and, as a result, induced the repair of membrane unity [42]. In another study, chitosan nanoparticles (~250 nm in size) loaded with levodopa (L-DOPA) showed no toxicity in PCL12 cells and significantly reduced caspase-3 gene expression compared to L-DOPA only [54]. Intravenously administered ferulic acid (FA) conjugated glycol chitosan nanoparticles (~240 nm in diameter) at 2 h after SCI protected axons and myelin at the pathological site and induced functional recovery by remaining in the systemic circulation for a long time [55].

3.3 Availabilities of Biocompatible Inorganic Nanostructures

Even though polymers have been studied extensively due to their advantages such as stability and degradability in biological fluids, it is difficult to control their size, shape, and biodegradation [56]. Inorganic nanoparticles have gained attraction as drug delivery vehicles because of their inert and biocompatibility properties, as well as their simple and controllable preparation and functionalization [57]. PEG coated gold nanoparticles (40 nm in size) delivered directly to the intraspinal space of mice reduced the inflammation that is mediated by microglia/macrophages, which play crucial roles in secondary injury [58]. Furthermore, remyelination was induced and motor function was enhanced via PEG-gold NPs [58].

Having larger surface area due to porosity makes mesoporous silica nanoparticles (MSNs) a potential cargo vehicles for delivery of neurotherapeutic agents [59, 60]. Bisperoxovanadium (BpV), which is a phosphatase and tensin homolog deleted on chromosome 10 (PTEN) inhibitor, was loaded into MSNs (<100 nm in size), and they were tested for their efficacy in axonal regeneration in-vitro and for internalization from various nerve tissues in-vivo [61].

Cerium oxide nanoparticles have been recently investigated for their free-radical scavenger activities [62, 63]. They have two oxidation states, which alternate between reduced (Ce^{3+}) and oxidized (Ce^{4+}) states [64, 65]. It was reported that ceria NPs prevent cell death from reactive oxygen species (ROS), which increase in brain after ischemic stroke [63, 66].

Titanium dioxide (TiO_2, titania) nanoparticles have been used in food, paint, cosmetics, pharmaceuticals and other consumer products as a whitening additive [67]. Biocompatibility, stability and inertness under physiological conditions make titania nanoparticles as potential drug delivery vehicle [68]. TiO_2 nanoparticle-doxorubicin drug delivery system showed improved anticancer efficacy and reduced adverse side effects as compared to the non-modified carriers [69]. In another study, high accumulation of daunorubicin within the cancer cells were observed in the presence of TiO_2 whiskers [70]. Further, the death of cancer cells were enhanced via photocatalytic activity of TiO_2 whiskers [70]. Silica/titania hollow nanospheres were loaded with camptothecin (CPT) and functionalized with herceptin for targeted breast cancer treatment [71]. Effective cellular internalization of CPT-nanoparticles and slow release of CPT was observed [71]. Another cancer drug, paclitaxel, was immobilized on PEG and folic acid functionalized TiO_2 nanoparticles [72]. Ninety percent of drug release was observed within 50 h with initial burst release followed by sustained release [72].

3.4 Our Biocompatible Titanate-Nanowired Hollow Nanospheres

Titanium dioxide nanoparticles have been mainly studied in cancer treatments; however, their effect on delivering neurotherapeutic agents has not yet been explored. Therefore, we prepared titanate hollow spheres and loaded with cerebrolysin (CBL), a brain-derived multipeptide drug, for the first time. The in-vitro release profiles and in-vivo efficacy of delivering cerebrolysin were investigated.

3.4.1 Hollow Titanate Nanosphere Synthesis

Hollow titanate nanospeheres (TiNS) were prepared according to the slightly modified literature procedures [73] (Fig. 2.1).

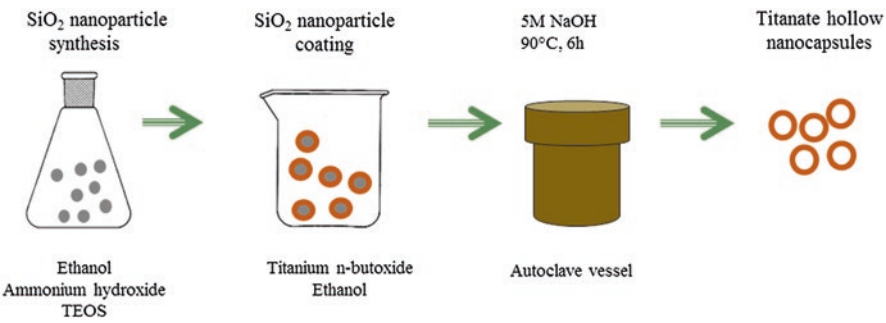


Fig. 2.1 The schematic synthesis of the hollow titanate nanocapsule

1. *Silica nanoparticle synthesis:*

Silica nanoparticles were used as a template to synthesize hollow titanate nanospheres. SiO_2 nanoparticles were created by the method established by Stober [1]. Briefly, solution A (ammonium hydroxide, ethanol, distilled de-ionized water) and solution B (TEOS and ethanol) were mixed, and magnetically stirred slowly for 6 h. The sample was washed with ethanol and DDI water three times alternating between sonicating and centrifuging for 15 min.

2. *Titanium oxide coating on SiO_2 nanoparticles:*

Silica nanoparticles were dispersed in ethanol and sonicated for 1 h. Titanium-n-butoxide (1:1 volumetric ratio to the amount of original TEOS in the sample) was added to the solution. The mixture was stirred vigorously for 4 h in a 45 °C water bath and sonicated for 60 min. The solution was then washed twice with ethanol and water to purify the coated nanoparticles. The final nanoparticles were dispersed in distilled water.

3. *Formation of the hollow titanate nanospheres:*

Titanium-n-butoxide coated SiO_2 nanoparticles (1 ml), sodium hydroxide (NaOH) (the final concentration of 5 M), and DDI water (9 ml) were mixed in a teflon liner and heated in an autoclave vessel in an oven at 90 °C for 6 h. After the process was done, the final particles were washed until they had a neutral pH.

4. *Cerebrolysin loading and controlled release studies*

Ten milligram of TiNS were soaked into cerebrolysin solution and left on orbital shaker for drug loading. Cerebrolysin loaded titanate nanospheres (TiNS-CBL) were incubated in 20 ml of phosphate buffer solution (PBS, pH 7.4) at 37 °C and were agitated at 120 rpm. At specific time points, the samples were centrifuged, and supernatants were collected. Cerebrolysin release from nanospheres was monitored over a period of 18 days. Absorbance values measured at 250 nm by UV-Vis spectrophotometer.

5. *Results from our investigations*

In our study, titanate nanospheres were prepared via templating method. Scanning electron micrographs showed uniform size distribution of silica nanoparticles at ~400 nm with smooth surface (Fig. 2.2). After hydrothermal reaction, titanium coating on silica spheres, the particle size increased to ~500 nm without changing its surface structure (Fig. 2.2). Silica templates dissolved by leaving porous titanate shells behind them after hydrothermal treatment of coated silica particles in alkali condition (Fig. 2.2). Furthermore, in-vitro release studies indicated that the 94% of the drug was released within 18 days. It was shown that sustained release of cerebrolysin was achieved.

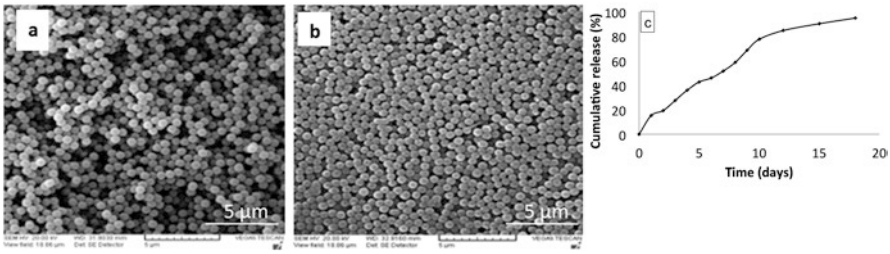


Fig. 2.2 Scanning electron micrographs of (**a**) silica nanoparticles; (**b**) coated silica nanoparticles, (**c**) cumulative release of cerebrolysin from the hollow titanate nanospheres

4 Nanofibers, Nanotubes, and Nanowires

The nanoparticles have attracted significant attention due to their smaller size, which has an effect on crossing the BBB, and their easy preparation [23]. However, nanocarriers can also be prepared in different shapes such as tubes, rods, fibers, belts, and wires [74, 75]. One of the advantages of creating tube- or rod-like structure rather than spherical morphology is creating a large axial-ratio, which creates large surface area that interacts with its surrounding environment [56].

4.1 Electrospun Nanofibers

Electrospinning is a widely used method for nanofiber production, and these nanofibers are either used as scaffolds and implanted to the defected area, or injected to the damaged site of the spinal cord [76, 77] (Fig. 2.3). Both natural polymers such as chitosan [78–80], collagen [81–84], or silk [85], and synthetic polymers such as poly(ε-caprolactone) (PCL) [86], poly(L-lactic acid) [87], or PLGA [88] can be electrospun into their corresponding solvents to form nanofibers. In a study, PC12 cells successfully differentiated into neural-like cells on electrospun polylactic acid/chitosan (PLA/CS) scaffolds under neurothrophin included media. Further, in-vivo studies presented that implanted PLA/CS scaffolds with neural-like cells prevented demyelination and axonal injury, which are the pathological hallmarks of multiple sclerosis (MS) [89]. In another study, 3-D hybrid scaffold composed of aligned poly(ε-caprolactone-co-ethyl ethylene phosphate) (PCLEEP) nanofibers, which were developed through electrospinning, and collagen hydrogel were implanted to the rats [90]. The results showed the fibrous scaffold that was implanted to the damaged spinal cord guided the axonal regrowth and induced the blood vessel formation without activating inflammatory response [90].

Gene delivery is also an important approach in nanotechnology. For this purpose, delivery of miRNAs—miR-219 and miR-338—via electrospun poly(ε-caprolactone) nanofibers, and their effect on differentiation and maturation of oligodendroglial

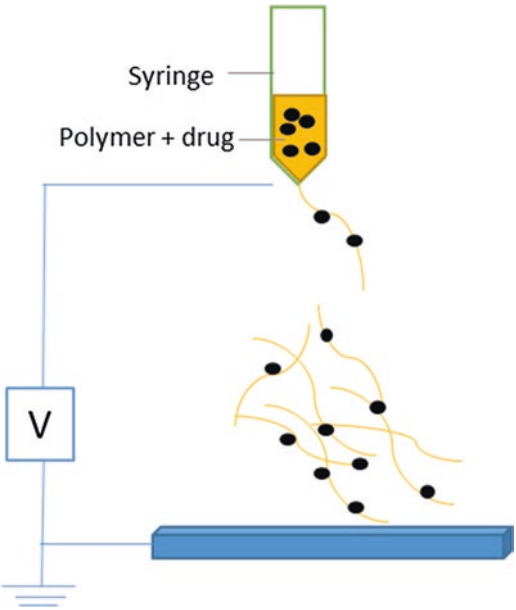


Fig. 2.3 Electrospinning method

precursor cells (OPCs) to oligodendrocytes, which are responsible from the formation of myelin in CNS, were investigated [91].

By controlling the spinning parameters such as solution parameters (e.g. molecular weight, concentration, viscosity, and conductivity of a polymer solution), processing parameters (e.g. voltage, flow rate, material of the collector, space between the syringe tip and the collector), and environmental parameters (e.g. temperature and humidity), it is possible to develop nanofibers with different morphologies and dimensions [92]. Additionally, nanofibers can be spun aligned or randomly-oriented [93]. It has been shown that both alignment and dimensions of nanofibers play a crucial role in neuronal regeneration [94, 95]. The good compatibility of nanofibers in cell growth is also attributed to the fibrillar structure similarity between native tissues and electrospun nanofibers [96].

4.2 Nanotubes

In addition to having large surface area similar to nanofibers, nanotubes also have large pore volume in which drugs can be stored at high concentration and delivered efficiently to the targeted location [56]. Nanotubes can be prepared from various materials, including lipids [97], proteins [98, 99], polymers [100, 101], and inorganic materials [102], by using different techniques, including physical/chemical template or directed/induced nanowire self-assembly [103].

Besides their applications in tissue engineering and gene/drug delivery, nanowires/nanotubes including platinum [104], silver [105], silicon [106–108] and titanium dioxide [109] have been studied as biosensors. It has been shown that coating neural electrodes with conducting nanotubes, which were prepared by deposition of conducting polymers, namely poly(3,4-ethylenedioxythiophene) (PEDOT) and poly(pyrrole) (PPy), on template PLLA nanofibers followed by PLLA fiber degradation, promotes cell adhesion and neurite outgrowth [110]. Significant decrease in the impedance and increase in the charge delivery capacity was observed in PEDOT nanotube coated gold electrode. Further, controlled release of dexamethasone from PEDOT nanotubes was demonstrated [111].

Carbon nanotubes are also of particular interests to researchers from biomedical field due to their superior structural, mechanical, and electronic properties [112]. Carbon nanotubes are discussed in Sect. 5.

Even though nanofibers/nanotubes have been studied in different medical fields such as tissue engineering, cancer, biosensing, imaging, etc., their use as drug delivery vehicles in SCI treatment has not been widely researched. Therefore, it is important to explore their roles in neuroprotection.

4.3 Our Titanate Nanowires

Given the fact that very limited research has been done on nanofiber/nanotube based drug delivery to the CNS, we have studied the effect of titanate nanowires as nanocarriers for neurotherapeutic agents.

4.3.1 Synthesis of the Sodium Titanate Nanowires

Titanate nanowires were prepared via a hydrothermal treatment of TiO_2 powder under high alkali conditions. Simply, 1 g of the TiO_2 powder (Degussa P25) was mixed with 40 ml of concentrated NaOH solution (10 M) and magnetically stirred for 10 min [113, 114]. The resultant mixture was placed into a Teflon-lined autoclave reactor and heated in an oven at 240 °C for 3 days. Thereafter, the product was washed with deionized distilled water until the pH reaches to 7. Between each wash, the samples were vortexed for 1 min and centrifuged at 4000 rpm for 3 min.

4.3.2 Preparation of the Hydrogen Titanate Nanowires

Prior to the intercalation, 2 g of the as-made sodium titanate nanowires were mixed with an NaCl solution (1 M, 150 ml), and the mixture was then kept under a mild magnetic stirring overnight. The product was then filtered into a large Buchner funnel, which was followed by washing with 0.1 M NaCl and deionized distilled water until pH 7 was reached [115].

For cation exchange, titanate nanowires were mixed with 1 M of HCl and allowed to stir for 3 days at room temperature. Nanowires were then filtered and repeatedly washed with deionized distilled water until pH was neutral. The product was dried under air and stored in a petri dish until further use.

4.3.3 Drug Loading

The hydrogen titanate nanowires were sterilized by soaking into 70% ethanol solution and phosphate buffered saline (PBS) prior to drug loading. Then, the nanowires were mixed with cerebrolysin (215.2 mg/ml), and stirred overnight at room temperature resulting in ~10 mg/ml of the nanowire-cerebrolysin solution.

4.3.4 Nanowired Drugs' Controlled Release In-Vivo

In early studies, our engineered titanate nanowires were tagged with various compounds other than cerebrolysin, and their neuroprotection effects were investigated in different CNS injury rat models [6, 116]. The results indicated that BBB and BSCFB permeability, and brain edema were significantly reduced with nanowired drug delivery. On the other hand, cognitive and sensory functions of rats were significantly improved based on their performances on rota-rod, inclined plane, grid walk, and foot print analyses [116–119].

Recently, cerebrolysin, a neurotrophic factor mixture, has also been loaded onto/into the nanowires and delivered to CNS in-vivo. The results presented increased neuroprotection effect on hyperthermia-induced brain damage compared to drug alone without nanowires [120].

4.4 Carbon Nanotubes

Carbon nanotubes (CNTs) are a durable, flexible, strong and electrically conductive carbon-based nanomaterial similar in size to neuronal processes [121]. They are cylindrical carbon molecules with novel properties applicable to many different fields, including drug delivery and neuroprotection. CNTs can be synthesized in varying sizes, ranging in diameter from 0.7 to 5 nm for single wall carbon nanotubes (SWNT), and from 2 to more than 30 nm for multi-wall carbon nanotubes (MWNT) [122].

Carbon nanotubes show promise in neuroprotection, neurodrug delivery, and neuroregeneration, consisting of the repair and regrowth of nervous tissues and the ability to interact with neurons [123]. Although they are insoluble in aqueous and physiological solvents, carbon nanotubes can be functionalized to increase their aqueous solubility. Carbon nanotubes can be used to deliver neurotrophins essential for development and function of neurons in both the central and peripheral nervous

system. They can also be conjugated with various other factors to promote targeting and administration of drug to various tissues such as certain areas of the brain, the spinal cord and neuronal tissues.

Carbon nanotubes have been found to promote tissue repair in spinal cord injury (SCI). Roman et al. discovered that SWNTs functionalized with polyethylene glycol (PEG) (SWNT-PEG) can be administered to promote regeneration and repair of a lesion post-SCI. Roman et al. created a rat model of SCI and analyzed the behavior of the rats before injury, before treatment with SWNT-PEG and each week for 4 weeks after treatment with SWNT-PEG at different concentrations. They found that issuance of SWNT-PEG to the SCI lesion decreased the overall volume of the lesion, increased the amount of fibers in the lesion, did not increase hypertrophy of glial cells, and caused a moderate amount of returned motion in the hind legs without increasing pain sensitivity. They concluded that administering SWNT-PEG prior to the SCI improves both the regeneration of the lesion and of the axons, suggesting that SWNT-PEG may be an effective nanomaterial for promoting recovery of tissue after SCI [124].

The application of CNTs for neuroprotection was studied by Hassanzadeh et al. The group tested the effects of MWNTs combined with anandamide (AEA), prepared using an amino-functionalized COOH-MWNT complex. Amino functionalization was found to improve the overall dispersibility of the CNTs and to reduce their toxicity. The amine-functionalized MWNTs were combined in solution with AEA to provide the CNTs with protective abilities. It was observed that the aminated MWNTs were promising carriers of AEA and the complex of the two could be a valuable therapeutic agent for neurodegenerative diseases such as ischemic stroke [125]. CNTs have also been found useful in restoring function to cells while performing as a neuroprotector. Xue et al. also found SWNTs to be useful as a neuroprotective approach to neurodegenerative diseases through an *in vitro* study of primary glial cells derived from mice. The carboxyl-functionalized SWNTs were found to restore normal autophagy function in primary glial cells with impaired autophagic degradation capacity. They also observed the reversion of impaired lysosomal proteolysis that causes buildup of the autophagic substrate and lysosomal swelling. The concentration of SWNTs used was very low to reduce the cytotoxic effect that is observed with CNTs [126].

Moon et al. experimented with CNTs as a means of delivery to promote repair of neural tissue after a stroke. The experiment was carried out *in vivo* using stroke damaged rats. The rats were transplanted with either hydrophobic (HP) or hydrophilic (HL) CNTs, controlled by the presence or absence of a hydrocarbon outer layer, respectively, which had been loaded with subventricular zone neural progenitor cells (SPZ NPCs). When compared to the rats transplanted only with SPZ NPCs or HL CNT-SPZ NPCs, the HP CNT-SPZ NPC transplant rats showed improved behavior and decreased volume of dead tissue. Overall, this study divulged that CNTs loaded with SVZ NPCs were able to restore stroke-damaged neuronal tissue, and that CNTs showed evidence of assisting in the differentiation of SVZ NPC into functioning neurons. The differentiation into functioning neurons might also aid in restoring the electrical activity of the stroke-damaged neural tissue [127].

Although they have shown potential for different neurological uses, the properties of CNTs make them toxic *in vivo*. Different effects of CNT toxicity have been observed, such as inflammation, cytotoxicity and tumorigenesis [128]. Liu et al. observed the changes in mice behavior after exposure to single walled carbon nanotubes (SWNTs), and found that SWNTs left the mice with reduced cognitive ability, hindered physical capability and left the brain damaged due to oxidative stress. The concentration of the SWNTs has an effect on the level of toxicity, and a form of protective functional group could potentially be used to reduce this toxicity [129]. Bosco et al. experimented with reducing the toxicity of CNTs by functionalization of polyethylene glycol. They infused polyethylene glycol-functionalized SWNTs (SWNT-PEG) into the rat hippocampus. After 7 days, the SWNT-PEG did not induce any damage to the hippocampus or leave the rats with any cognitive impairments. On the contrary, they actually observed formation of antioxidant defenses after a pro-oxidant effect that occurred immediately upon introduction of SWNT-PEG. At high initial doses, however, the SWNT-PEG displayed typical toxicity [130].

5 Nanocarrier Drug-Loading Strategies

Carrying drugs with nanostructures has been an important discovery for safe, sufficient and effective delivery to their targets. Development approaches have been mainly focused on protecting a drug from its physiological environment; however, drug loading is also an important parameter that needs to be considered for successful treatment [131].

Therapeutic agents can be embedded into the matrix, linked or absorbed to the surface or entrapped in the core of a nanocarrier [29, 32, 132]. Drug loading is performed either during or after nanocarrier synthesis, and it is closely related to nanocarrier fabrication, which affects the loading efficiency [131]. Solvent evaporation and its modified version—solvent diffusion—are commonly used techniques for nanosphere preparation [133]. These methods involve organic solvents such as chloroform or dichloromethane to dissolve polymers and hydrophobic drugs, and it is followed by oil-in-water (o/w) emulsion and organic solvent evaporation to produce particles [49]. Nanocarriers can also be formed via polymerization method in which monomers form a polymer [133]. In this technique, drugs can either be embedded into the matrix during polymerization process or attached on the surface after polymerization. Further, ionic gelation and coacervation methods are used to develop nanoparticle systems based on mixing two liquid phases that have electrostatic or ionic interactions, respectively [41, 42, 54, 133]. In the case of drug loading after nanocarrier synthesis, it is adsorbed on or trapped in the nanocarriers by soaking of nanoparticles in a concentrated drug solution or chemically conjugated to the surface of nanoparticles [55, 61] (Fig. 2.4).

High-drug loading is crucial for effective delivery of therapeutic agents, and it also depends on drug-material interactions such as surface charge, functional groups, or composition of either matrix materials or drugs [23]. For instance, it has

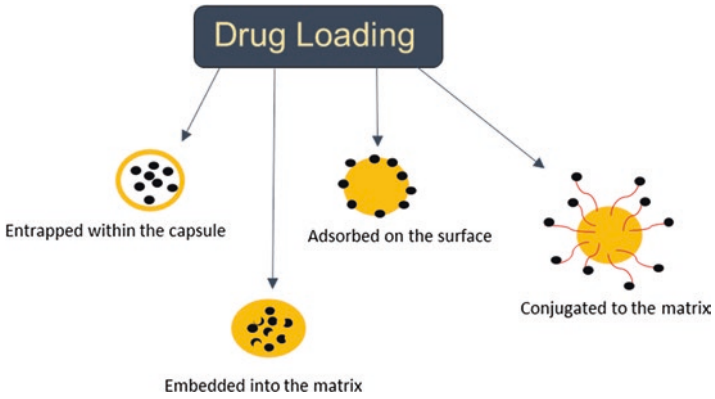


Fig. 2.4 Nanocarrier's drug loading

been shown that the highest loading efficiency is achieved when proteins are at or close to their isoelectric points [56, 134].

6 Future Directions

Significant studies have been conducted on the treatment of CNS injuries; however, current therapeutic limitations for treatment of nervous system degenerations make nanocarriers relevant as potential diagnostic and therapeutic drug delivery tools for neuroprotection. Nanocarriers are designed to protect drugs from their physiologic environments and to reduce their side effects, increase the circulation time, and provide sustained and controlled release of the agent at the defect site. Even though smart designs and successful advancements have been made for targeted nanoparticles to improve the treatment efficacy, only a few have reached the market. Abraxane, Emend, Doxil, and Abeicet are some of the examples for such products [135]. However, none of these nanotechnologically-formulated systems are used for neuroregeneration or neuroprotection.

Targeted delivery to the brain needs special attention due to its complexity in structure and diseases. An understanding of the roles of cells such as microglia, astrocytes, oligodendrocytes, endothelial cells, and neural stem cells in CNS pathology is needed to develop novel targeted-drug delivery systems. Along with specific targeting, more research should be done in determining the fate of nanocarriers regarding to their long-term toxicity, blood compatibility, and reticuloendothelial system (RES) elimination within physiological systems [136]. Remote-controlled drug delivery systems are also another promising approach for the development of multifunctional theranostic nanosystems. Triggering nano vehicles with external stimulants such as electro-magnetic field, photo irradiation, temperature, and pH may enhance their therapeutic efficacy at the targeted location.

By considering the factors discussed above, drug delivery combination with nanotechnology can be modified and improved to achieve safe, effective, and cost efficient therapy for clinical applications.

Due to the structural and functional limitations in CNS, conventional treatment methods create serious problems to patients or remain unsuccessful. Nano drug delivery strategies have potential in dealing with difficulties of drug transport and cell target. Even though, currently developed nanoparticle systems have not won the gold medal for successful treatment of CNS disorders, latest developments bring hope to the neuroscience field. Ultimately, the goal is to develop properly designed nanoparticles along with their optimization and evaluation. We believe that with the help of interdisciplinary collaboration, especially between nanotechnology and pharmaceutical industry, it is possible to develop clinically translatable drug delivery systems.

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