Nanotechnology for Blood–Brain Barrier Crossing

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Nanomedicine is currently focused on the design and development of nanocarriers that enhance drug delivery to the brain to address unmet clinical needs for treating neuropsychiatric disorders and neurological diseases. Polymer and lipid-based drug carriers are advantageous for delivery to the central nervous system (CNS) due to their safety profiles, drug-loading capacity, and controlled-release properties. Polymer and lipid-based nanoparticles (NPs) are reported to penetrate the blood–brain barrier (BBB) and have been extensively assessed in in vitro and animal models of glioblastoma, epilepsy, and neurodegenerative disease. Since approval by the Food and Drug Administration (FDA) of intranasal esketamine for treatment of major depressive disorder, intranasal administration has emerged as an attractive route to bypass the BBB for drug delivery to the CNS. NPs can be specifically designed for intranasal administration by tailoring their size and coating with mucoadhesive agents or other moieties that promote transport across the nasal mucosa.

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1. Polymeric NPs

Polymeric nanoparticles range in size from 1–999 nm. Synthetic polymers or copolymers of poly(D,L-lactic acid) (PLA), poly(ε-caprolactone) (PCL), PLGA, or natural polymers like chitosan and maltodextrins are used to formulate polymeric NPs. They are synthesised by the self-assembly of two or more chains of block copolymers with varying hydrophobicity using methods like solvent evaporation, nanoprecipitation, super critical fluid technology, and hot or cold homogenisation[1][2]. As PLGA is an FDA approved polymer, PLGA NPs have been extensively studied as drug delivery systems presenting many advantages; they easily cross the BBB, are biocompatible and stable, allow for controlled release kinetics, have high drug loading capacity, and can be functionalized with surface ligands for targeted drug delivery [3][4][5]. Furthermore, PLGA NPs are biodegraded by hydrolysis to produce lactic and glycolic acids, which enter the Kreb's cycle and are excreted as carbon dioxide and water [6]. Drug release occurs through bulk matrix degradation, however, many environmental factors like pH and the physicochemical NP characteristics can affect the rate of polymer degradation. Therefore, the release pattern is changeable but typically follows a biphasic profile [7]. Increases in PLGA NP size and concentration, as well as changes in shape, have been reported to cause cytotoxicity in vitro, resulting in macrophage activation and the production of reactive oxygen species (ROS). Nevertheless, the body of evidence suggesting that PLGA is

biocompatible far exceeds those that describe toxicity, and so, further studies are required to investigate physiological and toxicological responses to PLGA in vivo [8].

2. Solid Lipid Nanoparticles

SLNs are colloidal nanocarriers that range in size from 50–1000 nm. They are composed of solid physiological lipids, including phospholipids, triglycerides, fatty acids, and steroids, and can be prepared by high pressure homogenisation, ultrasonication/high speed homogenisation, and solvent emulsification/evaporation methods [9]. These preparation techniques have smooth scalability, reproducibility, and the manufacturing process does not involve toxic solvents [10]. Drug incorporation into SLNs can be in the form of a homogenous matrix, a drug-enriched core or a drug enriched shell. Release occurs by particle biodegradation by lipases, erosion, or diffusion, and is dependent on the lipid content, pH, temperature, and the drug entrapment model [11]. Properties of SLNs, such as high surface area and drug loading capacity, controlled release, improved stability, and long-shelf-life make them ideal drug carriers [12]. As SLNs are comprised of biological lipids, they are also biocompatible and easily cross the BBB [13]. Additionally, these lipids have a higher melting point than body temperature and remain in the solid-state post-administration [14][15]. SLNs have been developed and are being tested for many pharmaceutical applications, including the release of anti-tumour drugs like doxorubicin, tamoxifen, docetaxel, and methotrexate; drugs to treat high blood pressure like carvedilol; topical agents like tazarotene used in the treatment of skin conditions; anti-malaria medicine chloroquine; and antitubercular medications like isoniazid and rifampicin [15].

3. Surface Charge

The surface charge of NPs affects their cellular uptake, biodistribution, and fate in biological systems. Negatively charged NPs present a faster diffusion in tissues and a higher accumulation in tumour tissues when compared to positively charged NPs [16]. Due to favourable electrostatic interactions with negatively charged cell membranes, cationic NPs are more easily internalised by cells than neutral or anionic NPs. For this reason, positively charged NPs are more readily taken up by BBB endothelial cell membranes [17]. However, the feasibility of cellular uptake also results in the rapid clearance of cationic NPs from the circulation by macrophages. Additionally, increased liver accumulation is associated with positively charged NPs, which results in prompt plasma clearance and reduced bioavailability [18]. Positively charged NPs may also react with blood components causing haemolysis and toxicity [19]. Furthermore, cationic NPs have been shown to cause cytotoxicity and disrupt the integrity of the BBB, whereas such effects are not reported for neutral and anionic NPs [20]. The surface charge of NPs should be carefully considered in particle design and tailored specifically for the intended purpose. PLGA NPs and SLNs can be positive or negative depending on the synthesis method and may be altered by the surface chemistry.

4. Surface Modification

Surface engineering of PLGA NPs and SLNs can improve both biocompatibility, brain targeting, stability, and controlled drug release. Polymers like poly(ethylene glycol) (PEG), PCL, chitosan, and PEG-based surfactants like

polysorbate 80 and poloxamer 188 can be chemically grafted or adsorbed on the surface of PLGA NPs and SLNs. The hydrophilicity of these moieties increases steric hindrance and circulation time while prohibiting uptake by the reticuloendothelial system (RES) [21]. PEGylation of NPs for CNS drug delivery is common and is reported to improve the circulation time, biocompatibility, and brain uptake, even in pathological conditions[22][23][24][25]. Polymer coatings can also provide drug protection; for example, chitosan modification of SLNs protected against particle degradation at the acidic pH of the stomach following oral administration [26].

Proteins, aptamers, peptides, small molecules, and antibodies can also be conjugated to the surface of PLGA NPs and SLNs to improve drug targeting. CNS specific targeting can be achieved using ligands with high affinity for receptors and transporters expressed on the surface of BBB endothelial cells. These ligands include transferrin, lactoferrin, apolipoprotein E, glucose derivatives, and glutathione, which facilitate the brain uptake of NPs through receptor-mediated transcytosis and carrier-mediated transport mechanisms [21]. Cell-penetrating peptides (CPPs) like the transactivator of transcription can also be bound to the surface of NPs through covalent or non-covalent interactions[27]. Conjugation with CPPs can enhance transport through cell membranes, increasing BBB crossing and cellular uptake of drug-loaded NPs [28]. Furthermore, CPPs can overcome the p-glycoprotein (P-gp) efflux pumps expressed by BBB endothelial cells, which are associated with multi-drug resistance [29].

Particles can also be conjugated with mucoadhesive agents to facilitate nose-to-brain delivery. Chitosan, a bioactive polymer that improves cell penetration and has mucoadhesive properties, is a commonly used excipient for intranasal drug formulations and can be incorporated into the NP design for nasal delivery (for recent review of chitosan and its mucoadhesive properties, see Aderibigbe et al. (2019) and Mura et al. (2022) [30][31]). Chitosan electrostatically interacts with the negatively charged epithelial surfaces of the nasal cavity to enhance residence time and can also enhance penetration of cell membranes [32]. Additionally, this polymer absorbs water from the mucus lining the nasal cavity, causing the polymer to swell upon contact. This provides a greater surface area for drug crossing through the membrane and into the brain [33][34][35][36]. For this reason, numerous chitosan-based nasal formulations have been proposed as drug delivery systems to the CNS, including chitosan-dopamine and chitosan-tyrosine conjugates for PD [37], chitosan hydrogels for drug delivery in AD [38], chitosan-poloxamer gel for anti-epileptic drug (AED) delivery [39], chitosan nanoemulsions for glioblastoma multiforme (GBM) therapies [40], and chitosan-poloxamer nanoemulsions for the treatment of cerebral ischemia [41]. While NPs can be synthesised from chitosan, it is commonly used as a surface coating to enhance mucoadhesion and particle transport across the nasal mucosa and into the brain.

5. PLGA NPs and SLNs Are Compatible with Brain Cells In Vitro

To confirm the safety of PLGA NPs and SLNs in the brain microenvironment, both particle types have been studied in vitro for compatibility with neurons and other resident brain cells. PLGA NPs did not affect the integrity of human SH-SY5Y neuroblastoma cells, monocytes, and 16 HBE epithelial cells used to model the BBB, rodent PC12 catecholaminergic neurons, brain endothelial cells, primary microglia and primary astrocytes, or murine hippocampal neurons, N2a neuroblastoma cells, and N9 microglia [25][42][43][44][45][46][47][48]. Notably, prolonged

PLGA NP exposure did not alter neuronal morphology or affect the viability of primary rat neuronal-glial mixed cultures up to concentrations of 2.5 mg/mL [49]. Remarkably, 20 mg/mL PLGA NPs was not toxic to 16HBE cells [50]. Similarly, the application of SLNs to human hCMEC/D3 cerebral vascular endothelial cells, SH-SY5Y cells, primary rodent astrocytes, and brain endothelial cells or mouse BV-2 microglia, brain endothelial cells, and embryonic fibroblasts did not affect cell viability [13][51][52][53][54][55].

Furthermore, both PLGA and SLN nanosystems have been deemed compatible with various types of stem cell. The growth of mesenchymal stem cells on PLGA-based platforms was unaffected by the presence of polymeric structures [56]. In a study investigating the potential of SLNs to deliver neuronal differentiation factors to induced pluripotent stem cells (iPSCs), SLNs were non-toxic to stem cells [57]. Flow cytometry revealed no difference in the number of live cells when a human iPSC-based BBB model was exposed to 50 and 100 nm PLGA NPs for 20 h [58], highlighting the potential for the safe translation of these nanocarriers to the clinic for drug delivery to the CNS.

6. Permeation of In Vitro BBB Models

In vitro models have been established to confirm the ability of PLGA NPs and SLNs to cross the BBB. Cells that make up the BBB can be cultured in a monolayer on transwell devices so that following the application of NPs, the percentage that pass through the cell layer into medium on the basolateral chamber can be quantified (for review of in vitro BBB models, see Williams-Medina et al., 2020 [59]). The modification of PLGA NPs with lactoferrin or anti-transferrin receptor monoclonal antibody increased BBB crossing in vitro [45][60]. Similarly, SLNs effectively crossed cerebral vascular endothelial cells and conjugation with apolipoprotein E or transferrin significantly increased cell uptake [13][51]. In a multicellular BBB model consisting of primary rat brain endothelial cells, astrocytes, and pericytes, SLNs penetrated the barrier and targeting was increased over 3-fold by surface modification with apolipoprotein E [54].

7. PLGA NP and SLN Drug Delivery to In Vitro CNS Disease Models

Prior to in vivo evaluation, PLGA NP and SLN drug delivery vehicles have been evaluated in in vitro models of neuroinflammation, neurodegeneration, and brain cancers to assess drug release and drug action.

7.1. Neurodegenerative Disease

In vitro models of neurodegeneration can be achieved by applying disease salient factors to brain-derived cells. Insights into the in vivo efficacy and therapeutic doses of substances released from PLGA NPs and SLNs can be gained through in vitro screening. PLGA-PEG NP delivery of fucoxantin, a marine carotenoid that is reported to have neuroprotective effects, prevented Aβ-induced neurotoxicity, ROS production, and the release of pro-inflammatory cytokines in SH-SY5Y and BV-2 microglia cells [61]. Pre-treatment with resveratrol-loaded PLGA NPs inhibited H_2O_2–induced ROS production and was protective against 1-methyl-4-phenylpyridinium (MPP^+)-induced mitochondrial dysfunction and cytotoxicity in SH-SY5Y cells as an in vitro model of PD [45]. Similarly, the concurrent

application of drug-loaded SLNs with 6-hydroxydopamine (6-OHDA)-induction of an SH-SY5Y cell model of PD was cytoprotective [52]. SLNs also successfully delivered anti-inflammatory therapies to lipopolysaccharide (LPS)-stimulated microglial cells, attenuating nitric oxide production, the expression of nitric oxide synthase and cyclooxygenase-2 (COX-2), and the production of pro-inflammatory cytokines [53]. The release of idebenone, an anti-oxidant agent, from SLNs was protective against 2,2′-azobis-(2-amidinopropane)dihydrochloride-induced oxidative stress in primary rat astrocytes, as measured by a reduction in cytotoxicity and the production of ROS [55].

7.2. Brain Cancer

Robust in vitro models of brain cancers exist, which involve culturing tumour cells and testing drug efficacy by measuring cell death. PLGA NPs loaded with a derivative of the anti-cancer drug temozolomide were non-toxic to 16HBE cells but reduced the viability of T98G GBM cells to 20% of control [46]. Doxorubicin-entrapped SLNs induced cell death when applied to U87MG GBM cells [62]. Furthermore, PLGA NPs conjugated with an anti-epidermal growth factor receptor (EGFR) monoclonal antibody and loaded with curcumin achieved a reduction in the growth of EGFR-expressing GBM cells at lower concentrations than those required for free curcumin or unmodified curcumin-loaded PLGA NPs to achieve this effect [63]. Lipid-based and polymeric NPs are also being explored for the delivery of chemotherapeutic agents in paediatric cancers (for review see Guido et al., 2022 [64]).

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