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Governing transport principles for nanotherapeutic application in the brain

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Neurological diseases account for a significant portion of the global disease burden. While research efforts have identified potential drugs or drug targets for neurological diseases, most therapeutic platforms are still ineffective at reaching the target location selectively and with high yield. Restricted transport, including passage across the blood–brain barrier, through the brain parenchyma, and into specific cells, is a major cause of ineffective therapeutic delivery. However, nanotechnology is a promising, tailorable platform for overcoming these transport barriers and improving therapeutic delivery to the brain. We provide a transport-oriented analysis of nanotechnology's ability to navigate these transport barriers in the brain. We also provide an opinion on the need for technology development for increasing our capacity to characterize and quantify nanoparticle passage through each transport barrier. Finally, we highlight the importance of incorporating the effect of disease, metabolic state, and regional dependencies to better understand transport of nanotherapeutics in the brain.

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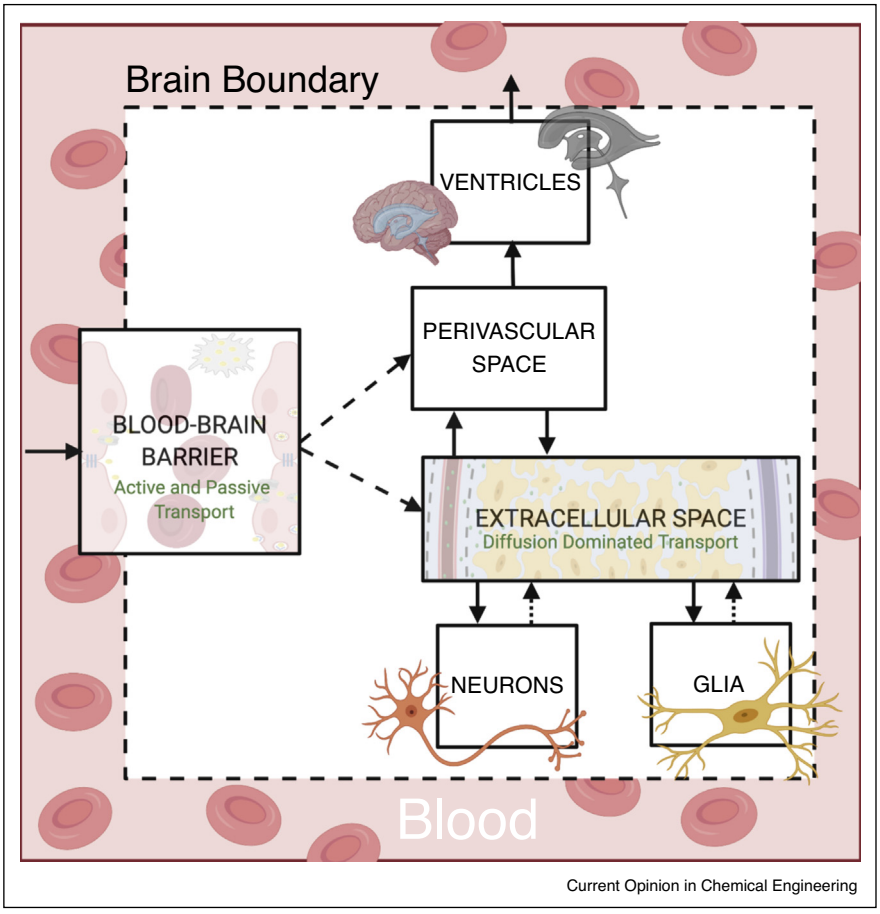
Introduction

Neurological diseases, including Alzheimer's, Parkinson's, stroke, and traumatic brain injury, are the leading cause of disability-adjusted life years and the second leading cause of death globally [1]. Many targets for neurological diseases have been identified for therapeutic intervention [2,3], but due to the complexity of transport in the brain, systemic-based delivery approaches are largely ineffective [4,5]. Therefore, yield, accumulation, and selectivity of administered neurotherapeutic drugs at specific targets within the diseased brain are a technological area of emphasis for improvement.

To increase efficacy of neurotherapeutics, researchers are improving the transport of drugs to and within the brain. While the scope of this perspective focuses on transport from systemic (vascular) flow, alternative administration routes are viable options. Examples of alternative administration routes are intraparenchymal, intranasal, and intraventricular, which avoid first-pass metabolism [6] and bypass the blood–brain barrier (BBB). Recent reviews provide a comprehensive analysis on effect of administration route on therapeutic delivery and efficacy [7,8]. From the viewpoint of systemic administration, nanotherapeutic delivery historically views the brain as a 'black box', where the input is the advection of a therapeutic platform in blood to the BBB, and the output is waste removal through the perivascular network during cerebral spinal fluid fluxes [9]. Most neurotherapeutics must overcome the BBB as part of the neurovascular unit, navigate the brain parenchymal space, and uptake or act on specific cells. Traversing these three complex barriers necessitates neurotherapeutic platforms with a large design space. Because of their high tailorability, nanoparticles are a promising platform for effective drug delivery to the brain.

There are many design options to consider when selecting and modifying nanoparticle-based therapeutics for brain diseases. However, design approaches often focus on overcoming the BBB while overlooking other transport complexities within the brain environment [5,10]. We provide a transport-oriented view for improved nanotherapeutic design to increase selectivity, yield, and accumulation of therapeutics at disease sites in the brain. We evaluate transport at and between three major barriers: (1) across the BBB, (2) through the parenchyma, and (3) into target cell types (Figure 1). Additionally, we discuss that any nanotherapeutic system needs to be tailored to navigate or overcome changes in the brain due to disease, metabolic states, and regional dependencies [11•,12].

Figure 1



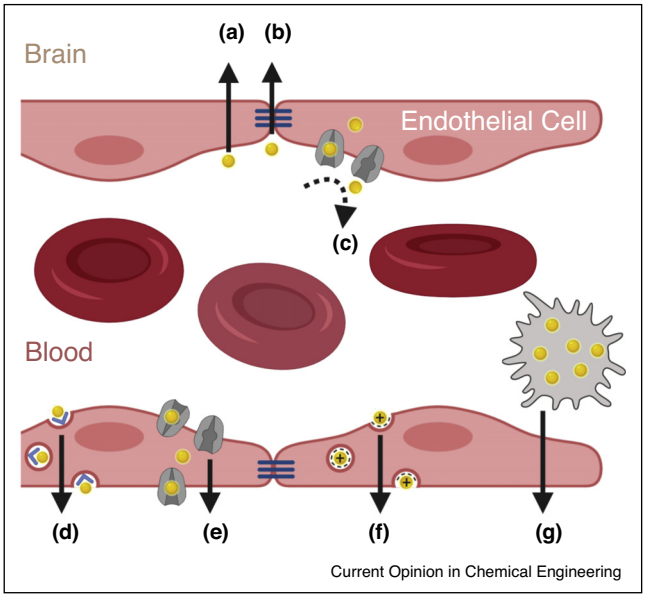
Block flow diagram of nanotherapeutic transport in the brain.
After systemic administration, nanotherapeutics enter the brain through the BBB. From the BBB, nanotherapeutics enter the perivascular or ECS. From the perivascular space, nanotherapeutics either exit the brain through the ventricles or move to the extracellular space. From the ECS, neurons or glia can internalize the nanotherapeutic. [Created with BioRender.com].

Engineering transport across the blood–brain barrier

Nanoparticle transport across the BBB can be organized into two categories: passive and active (Figure 2). Passive transport includes diffusion of aqueous nanoparticles in the confined space between brain capillary endothelial cells (BCECs) and the more common diffusion of lipophilic nanoparticles through the BCEC plasma membrane. Active transport can be mediated by various receptor-mediated, carrier-mediated, adsorptive-mediated, or cell-mediated pathways for endocytosis, transcytosis, and efflux [13]. Examples of nanoparticles which can leverage each route are discussed in recent reviews [13,14]. To enhance transport across the BBB, nanoparticle surfaces may be engineered to leverage one or more active targeting strategies. Transport may also be enhanced by optimizing treatments or timing for specific BBB pathologies. For example, in models of adult stroke, BBB breakdown is biphasic, with acute disruption 3–5 hours after injury and more widespread loss of barrier function 48 hours after injury [15]. In other neurological disorders, BBB breakdown can be continuous or monophasic, and generally the relationship between disease severity and BBB pathology is poorly characterized [11•]. This characterization should optimally be conducted at both the whole-organ scale and at the cellular level in order to identify specific transport pathways and receptor kinetics which may be more or less vulnerable after injury.

Unfortunately, measurement of nanoparticle transport across the BBB is limited by the absence of standard techniques to characterize BBB integrity and passage. Evans Blue (EB) is the most common fluorescent chemical marker used to assess BBB integrity, but EB has *in vivo* limitations including limited stability in saline, toxicity at commonly administered doses, and nonspecific serum protein binding [16]. Instead, using a combination of differently sized dextrans or radiolabeled molecules is

Figure 2



Schematic of nanoparticle transport routes across the BBB. Nanoparticles (yellow) can passively diffuse **(a)** through endothelial cell membranes, or **(b)** between endothelial cells. Alternatively, nanoparticles can be actively **(c)** effluxed or internalized via: **(d)** receptor-mediated, **(e)** carrier-mediated, **(f)** adsorptive-mediated, or **(g)** cell-mediated endocytosis and transcytosis. [Created with BioRender.com].

recommended [16]. Even so, quantifying nanoparticle amount in brain tissue after systemic administration remains a challenge. Typically, brain tissue is homogenized at set timepoints after nanoparticle administration and the homogenate is measured for fluorescence or radioactivity of the administered drug or nanoparticle. However, homogenization and analysis of the whole brain do not separate nanoparticles which were able to cross the BBB from those accumulated in the luminal or perivascular spaces [11•]. While perfusing vessels can help, perfusion flow rates must be rigorously controlled to minimize further influence on nanoparticle distribution. Techniques to isolate microvessels by capillary depletion can provide more sensitive characterization of nanoparticles that permeated into the brain parenchyma [17]. Overall, more rigorous methods need to be adopted as field standards for the quantification of nanoparticle transport across the BBB.

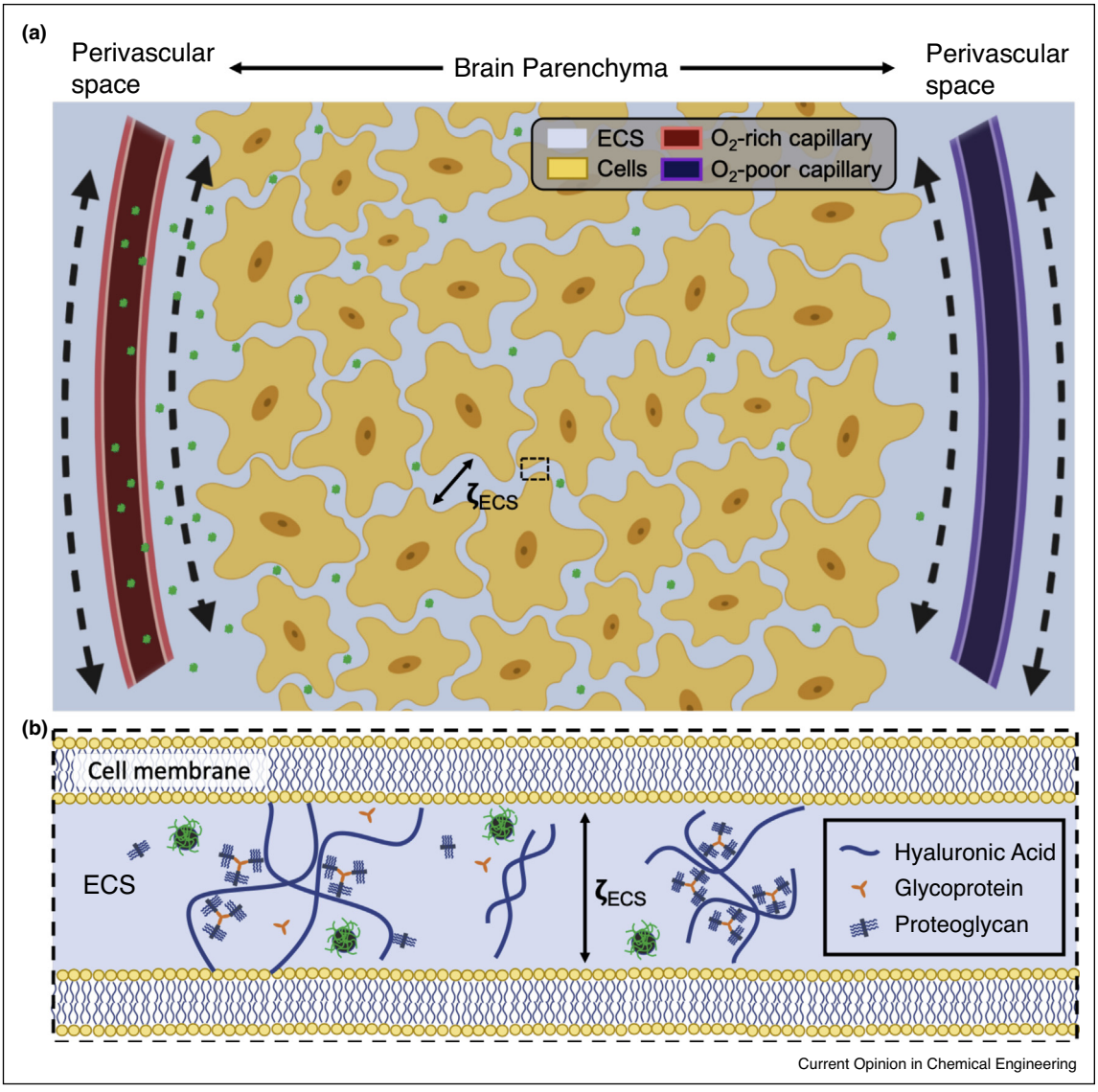
Investigators can take advantage of recent technological breakthroughs to conduct higher-throughput or more mechanistic evaluation of nanoparticle transport across the BBB. For many years, membrane co-cultures of animal-derived BCECs, astrocytes, and/or pericytes have been used to recapitulate key physiological characteristics of the BBB. However, inevitable species differences limit the translational potential of these models. Lippmann *et al.* addressed this challenge in 2012 by developing a BBB model with brain microvascular endothelial cells (BMECs) derived from human-induced pluripotent stem cells (hiPSCs) [18]. The hiPSC-derived BMECs express BBB markers while exhibiting tight barrier properties and functional transport systems. In 2019, the Wyss Institute published a BBB Chip with hiPSC-derived BMECs, astrocytes, and pericytes, which recapitulated a high level of barrier function for an extended period of at least one week *in vitro* [19•]. More recently, Ahn *et al.* used a similar microphysiological platform to evaluate nanoparticle distribution at the cellular level and identify receptor-mediated transcytosis as a mechanism of nanoparticle uptake [20••]. Tools like these hold significant potential to screen many nanoparticle platforms for understanding BBB transport properties.

Nanoparticle transport within the brain parenchyma

Nanotherapeutics that cross the BBB and access brain parenchyma are subject to a heterogeneous environment filled with cellular bodies, cell processes, and brain extracellular matrix (ECM). Nanoparticles must navigate the narrow channels of the ECM-filled brain extracellular spaces (ECS), which are 5–36% (~19% average) of healthy brain volume [21]. Movement in the ECS is potentially driven by a combination of diffusive and convective transport; however, the existence of bulk interstitial flow remains a debated topic in the field [22,23••]. The development of enhanced techniques for real-time and fast assaying of interstitial fluid flow *in vivo* will be essential to bridging this divide.

Regardless, diffusion-driven transport undoubtedly occurs in brain parenchyma. Nanoparticle diffusion is influenced by both ECS geometry and interactions with brain ECM. The highly tortuous geometry (Figure 3a) increases the mean free path a nanoparticle must travel, and the presence of dead space domains leads to the transient trapping of particles, increasing residence time in specific brain compartments and reducing particle flux [24]. Nanoparticles are also subject to viscous drag brought about by the finite width of the ECS and pores that exist in brain ECM. Until 2012, all ECS channels were believed to fall below a width (ζ_{ECS}) of 64 nm, based on biophysical studies that tracked the diffusion of polyethylene glycol (PEG)-coated quantum dots (QD) in rat brain *in vivo* [25]. However, Nance *et al.* demonstrated that probes used in previous studies suffered from an insufficient surface coating of PEG, exposing surface binding domains on the QD. A denser PEG coating allowed nanoparticles as large as 114 nm in diameter to diffuse effectively within human, rat, and mouse brains [26]. Similar studies have since confirmed this, and the majority of ECS widths (ζ_{ECS}) are now thought to exist between 80 and 220 nm with >25% of all pores ≥100 nm [26–28,29••]. Even then, these more recent size range

Figure 3



Schematic of nanoparticle transport within the brain ECS.
(a) Nanoparticles (green) able to cross the BBB can be transported via convection along the perivascular spaces of the neurovascular unit, as indicated by the dashed arrows. Arrows are bidirectional as the directionality of this flow remains an ongoing debate. Nanoparticles can also access brain parenchyma, where diffusion down a concentration gradient drives transport through the highly tortuous brain ECS. **(b)** Diffusion is hindered by the presence of brain ECM, which forms three-dimensional mesh-like structures out of hyaluronic acid, glycoproteins, and proteoglycans. [Created with BioRender.com].

predictions are still potential underestimates. While studies have demonstrated the ability of PEG to limit nanoparticle uptake into brain cells [30], definitively labeling a nanoparticle as extracellular during a particle tracking experiment remains challenging. Any internalized nanoparticle exhibiting confined diffusion would lead to smaller predictions of local pore size. Regardless, the discoveries made by Nance and others are significant because the width of the ECS places an effective upper limit on the hydrodynamic size of nanoparticles able to diffuse in brain tissue.

The necessity of a dense inert surface coating introduces the potential for nanoparticle interactions with the brain ECM. Brain ECM consists predominantly of anionic glycosaminoglycans that can be free floating, tethered to cellular surfaces, or condensed to form tight meshes that sheathe certain cell populations (Figure 3b). ECM

structure and composition can alter interstitial fluid viscosity and regulate the width and geometry of local ECS [28,31]. The highly anionic composition can repel or attract any charged substances and cause deviations from normal diffusion [32]. Hydrophobic interactions between nanoparticle surfaces and ECM proteins can also play a role, as shown by experiments that studied the effects of altering either the surface chemistry of the nanoparticle probe [26,27] or chemically altering the composition of brain ECM [28]. However, the extent to which individual ECM-related factors contribute to hindered extracellular diffusion is difficult to quantify. We need further development of techniques capable of isolating individual factors within an *in vivo* brain microenvironment to better direct the design of nanotherapeutics to overcome parenchymal transport barriers for improved therapeutic efficacy.

Selective nanoparticle transport into brain cells

It is important to understand the parameters that influence nanoparticle transport or cellular uptake into target brain cells to improve therapeutic outcome. The targeted cell type is often selected based on the disease of interest or mechanism of action of the therapeutic payload (Table 1). Nanoparticle physicochemical properties, including size, shape, surface charge, and surface chemistry, mediate the interaction between brain cells and nanoparticles [33]. Disease state [34], activated status of individual cells [35,36], or the age of cells [37] also drive cell-nanoparticle interaction. Additionally, these parameters can be interdependent. For example, a recent study of nanoparticle axonal retrograde transport reveals transport mechanisms depend on cell type, particle charge, and particle intracellular internalization into lysosomes [38]. In addition, nanoparticle surface chemistry can alter colloidal stability and surface charge, which both influence the cellular fate and cellular uptake mechanism [39•,40].

Although brain cell-nanoparticle interactions have been well-studied, contradictory findings for individual cell-nanoparticle interactions persist in the field. These contradictions are likely due to the variability of models used in each study, which can have differing transport barriers that affect nanoparticle uptake into cells. For example, *in vitro* mono-cultures lack the representation of complex multi-cellular networks and ECM, resulting in eventual non-specific uptake of nanoparticles into many cells, especially with higher doses or longer exposure times [41]. *In vitro* mixed primary cultures are more complex but lack ECM. *Ex vivo* organotypic brain slices provide 3D architecture of cells and ECM and can preserve regional differences within the brain; however, vascular and ventricular flow effects are absent. Our group previously demonstrated the importance of model choice for understanding nanoparticle-cell uptake. For example, PEGylated QDs penetrate well into brain slices, while non-PEGylated QDs rapidly aggregate, which prevent further transport [39•]. This result was not seen in monolayer cell culture due to the absence of the parenchymal barrier. *In vivo* models provide the BBB, fluid flow and solute exchange, yet are difficult to probe transport mechanistically at spatial and length scales relevant to real-time nanoparticle behavior.

At first glance, the limitations of each model might drive the need to use multiple models to study the cellular uptake of individual nanotherapeutic platforms. However, recent findings show it is possible to use a singular model by accounting for transport barriers present or absent. One study showed the fate of a particle introduced into the brain interstitium is mainly determined by three rate-driven phenomena: the cellular association rate (k_{on}), the clearance rate due to exchange of fluids (k_{CSF} and k_{sys}), and the cell division rate (k_{mit}) [40]. This study demonstrated the association rate measured *in vitro* in cultured cells can predict the extent of internalization *in vivo*. To extrapolate this work further, other factors that can affect nanotherapeutic cellular uptake must be incorporated into the model, including dose and treatment time [42]. The presence of serum, which can drive the formation of a protein corona, can affect nanoparticle cellular uptake [30,43]. Additionally, the effect of cell sex [44••], metabolic state, and cell phenotypic and functional subtypes are current gaps in our understanding of transport-mediated processes that drive brain cell-nanoparticle interaction. Emerging and alternative technologies, such as the lab on a chip [20••], as well as integration of *in vitro* to *in vivo* models with computational simulation, will continue to increase our knowledge of cell-nanoparticle interactions in the brain.

Concluding thoughts: looking forward

For effective neurological disease treatment, nanotherapeutics must be designed to successfully transport across the BBB, through the ECS, and into target cells. Successful neurotherapeutic transport must also account for regional dependencies, metabolic demand, and disease state. Regional dependency adds transport complexity to the ECS. For example, tissue stiffness differs between white and grey matter due to myelination during development [6]. Differences in tissue stiffness and cellular composition alter rate of diffusion through the ECS, affecting access of nanoparticles to target cells. Alongside regional differences, variable metabolic demand of the brain increases transport complexity. When the body undergoes changes in metabolic activity, cerebral blood flow and subsequent cerebral oxygen consumption in the brain is altered [45]. Energy demand during sleep or under the effects of anesthesia have specifically been shown to cause alterations to fluid flow, solute efflux, and cell metabolism [12,46,47]. These alterations affect the rate of uptake pathways across the BBB, diffusion barriers

Table 1

Overview of nanotherapeutic-cell interaction in the brain sorted by cell type and described by cell function, therapeutic targets, nanoparticle platforms, and cell uptake mechanisms. Nanoparticles are abbreviated, and where applicable, described by the following representative symbols: antibody conjugated ceria-zirconia nanoparticles (7CZ-Ab, ∑); calcium phosphate lipid coated nanoparticles (CaP-Liquid, ±); carbosilane dendrimers (D-carbosilane, §); copper oxide nanoparticles (CuO, £); cyclodextrin nanoparticles (CDP, β); dendritic polyglycerol sulfate nanoparticles (dPGS, θ); gold nanoparticles (Au, †); HDL-mimetic nanoparticles with apolipoprotein A1 (eHNP-A1, †); mesoporous silica nanoparticles (MSNs, D); nanodioamonds (NDs, ◇); polyamidoamine dendrimers (D-PAMAM, ‡); PLGA conjugated with transferrin and BSA (Tf-PLGA, π); PLGA-PEG conjugated with lactoferrin (Lf-PLGA-PEG, σ); poly(methoxypolyethyleneglycol cyanoacrylate-co-hexadecylcyanoacrylate) (PEG-PHDCA, Ψ); polysorbate 80-coated polybutylcyanoacrylate nanoparticles (P80-PBCA, Ω); poly(lactide-co-glycolide) nanoparticles (PLGA, □); polystyrene nanoparticles (PS, ○); quantum dots (QDs, λ); silicon nanoparticles (SNPs, α); silver nanoparticles (Ag, ε); superparamagnetic iron oxide nanoparticles (SPIO, η); titanium dioxide nanoparticles (TiO2, Δ); trimethyl chitosan nanoparticles (TMC, ℓ); water-soluble upconversion nanoparticles, based on polyvinylpyrrolidone (PVP)-coated NaYF4:Er3+,Yb3+,Gd3+ (UCNPs, ø)

Cell type	Microglia	Neurons	Astrocytes	Oligodendrocytes	Endothelial cells
Function	Native immune cells in the brain	Electrically excitable cells that communicate with other cells via synapses	Glial cells that provide biochemical support of endothelial cells of BBB	Cells that provide support and electrical insulation to the axons of neurons in the brain	Cells that form the BBB
Therapeutic target	Alzheimer's disease	Alzheimer's disease	Alexander's disease	Alzheimer's disease	Alzheimer's disease
	Autism spectrum disorders	Parkinson's disease	Alzheimer's disease	Autism spectrum disorder	Parkinson's disease
	Huntington's disease	Huntington's disease	Autism spectrum disorders	Schizophrenia	
	Ischemic stroke	Ischemic Stroke	Huntington's disease	Ischemic stroke	
	Multiple sclerosis	Schizophrenia	Ischemic stroke		
	General neuropathic pain				
	Rett syndrome				
Nanoparticle platform	7CZ-Ab	Ag	Ag	D-PAMAM	Ag
	Ag	Au	CuO	MSN	Au
	Au	D-PAMAM	D-carbosilane	QD	eHNP-A1
	CDP-NO	MSNs	D-PAMAM	SPIO	Liposomes
	D-PAMAM	NDs	dPGS		PBCA
	dPGS	PLGA	MSN		PEG-PHDCA
	PLGA	PS	QD		PLGA
	QD	QD	SPIO		PLGA-PEG
	SNP	TMC	TiO_2		
	SPIO		UCNPs		
	TiO_2				
Uptake mechanism – particle type superscripted	Clathrin-dependent and/or caveolin-dependent endocytosis$^{\alpha,\lambda,\ddagger,\Delta,\eta,\theta,\varepsilon}$	Clathrin-dependent and/or caveolin-dependent endocytosis$^{\ddagger,\square,\diamond,\pm}$	Endocytosis$^{\Delta,\eta,\varepsilon,\text{ø}}$	Endocytosis$^{\eta}$	Clathrin-dependent endocytosis$^{\Psi,\sigma}$
	Macropinocytosis$^{\alpha,\lambda,\ddagger,\eta,\varepsilon}$	Phacogytosis$^{\varepsilon}$	Macropinocytosis$^{\eta,\varepsilon}$		Mediated by scavenger receptor class B type 1 (SR-B1)†
	Microglia-specific receptor (mannose) endocytosis$^{\lambda}$		Phagocytosis$^{\varepsilon}$		Phagocytosis$^{\Omega}$
	Phagocytosis$^{\alpha,\lambda,\Delta}$				Receptor-mediated endocytosis$^{\Omega,\pi,\sigma}$
	Receptor-mediated endocytosis$^{\sum}$				

through the ECS, and uptake mechanisms into target cells.

Finally, transport within the brain is affected by disease state. Disease generally disrupts BBB integrity and permeability, reconfigures the microenvironment, and changes the functional or metabolic state of target cells. In Alzheimer's and Huntington's disease, glucose transport is decreased across the BBB and into target cells, which hinders the trafficking of nanotherapeutic targeted for the glucose pathway in these diseases [48,49]. While this is just one example, many nanoparticle applications

in various animal models of brain disease are recently reviewed to highlight the disease-dependent nature of nanoparticle transport in the brain [50].

Nanoparticle platforms must be engineered with a transport-oriented mindset to increase yield and selectivity at the target destination. Well-designed nanotherapeutics have properties that utilize selective transport across the BBB, effective diffusion through the ECS, and result in specific cell uptake. Importantly, there is a need for tools that can increase our capacity to: (1) standardize metrics for quantifying and capturing nanoparticle-BBB passage in real-time; (2) identify spatially and temporally accurate access to the target sites of therapeutic action, and the barriers that exist between the entry point in the brain and those target sites; (3) isolate contributing factors to hindrance of nanoparticle transport within the brain parenchyma, while retaining *in vivo* flow and cell architecture; and (4) generate reproducible results that are translatable across models and that account for biological variables like cell sex. The development and implementation of these tools will not only improve our understanding of transport in the brain environment across many biological states, but it will also drive the development of robust and intentionally designed nanotherapeutics for the treatment of the diseased or injured brain.

Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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