

Micro/nanosystems for controllable drug delivery to the brain

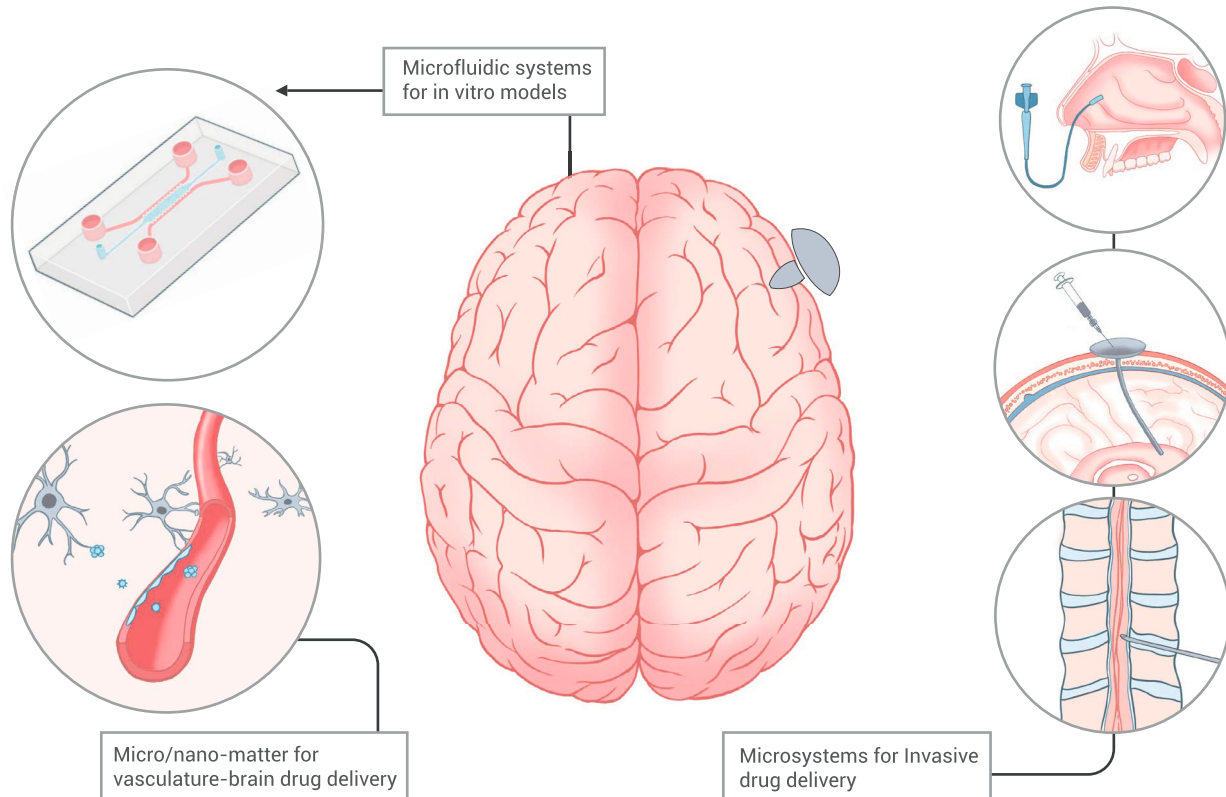
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GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Micro/nanosystems show their potential to address the challenges of precise drug delivery to the brain.
- Microfluidic platforms enable the creation of biomimetic *in vitro* brain models.
- Micro/nano materials is emerging as a key player in controllable brain drug delivery.
- The minimally invasive fiberbot microsystem reduces the procedure's invasiveness.
- Image tracking of micro/nanosystems allows for controlled therapeutic interventions.



Micro/nanosystems for controllable drug delivery to the brain

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Drug delivery to the brain is crucial in the treatment for central nervous system disorders. While significant progress has been made in recent years, there are still major challenges in achieving controllable drug delivery to the brain. Unmet clinical needs arise from various factors, including controlled drug transport, handling large drug doses, methods for crossing biological barriers, the use of imaging guidance, and effective models for analyzing drug delivery. Recent advances in micro/nanosystems have shown promise in addressing some of these challenges. These include the utilization of microfluidic platforms to test and validate the drug delivery process in a controlled and biomimetic setting, the development of novel micro/nanocarriers for large drug loads across the blood-brain barrier, and the implementation of micro-intervention systems for delivering drugs through intraparenchymal or peripheral routes. In this article, we present a review of the latest developments in micro/nanosystems for controllable drug delivery to the brain. We also delve into the relevant diseases, biological barriers, and conventional methods. In addition, we discuss future prospects and the development of emerging robotic micro/nanosystems equipped with directed transportation, real-time image guidance, and closed-loop control.

INTRODUCTION

The global burden of brain diseases is on the rise, making them a significant cause of disability and mortality worldwide.¹ As the world is transitioning into an aging population, the prevalence of neurodegenerative disorders including Alzheimer's disease (AD), Parkinson's disease (PD), and cerebrovascular diseases like stroke, are posing increasing threats to the society. In addition, mental illnesses have recently gained recognition as substantial contributors to the overall global disease burden.² Furthermore, primary brain tumors and brain metastases persist as conditions universally associated with high fatality rates.³ Accidents are also a common cause of brain trauma and spinal cord injuries. Moreover, rare diseases, including those affecting the brain such as amyotrophic lateral sclerosis (ALS), have recently garnered substantial attention. Consequently, the imperative of developing drugs with enhanced transport and targeting efficiency for brain-related conditions has come to the forefront. Unfortunately, the commercial development of drugs for brain diseases faces significant obstacles, characterized by protracted research and development timelines and elevated rates of failure.⁴ This predicament primarily emanates from challenges inherent in delivering drugs to the brain, notably biological barrier limitations.⁵ Micro/nanosystems offer promising alternatives for facilitating drug delivery to the brain due to their small size and precise manipulability. Moreover, the deployment of *in vitro* human brain models fabricated utilizing micro/nanosystems, furnishes robust tools for drug screening, personalized treatment strategies, and pathological studies.⁶

This review seeks to offer a comprehensive overview of the challenges associated with drug delivery for brain diseases and the emerging solutions that capitalize on the advancements in micro/nanosystems. First, we focus on the utilization of micro/nanosystems, with a particular emphasis on microfluidic systems, for the fabrication of *in vitro* brain models, encompassing both artificial and self-forming structures. Subsequently, we systematically explore micro/nano drug delivery systems, covering both non-invasive vascular-brain delivery approaches and invasive methods. In addition, we present the current state-of-the-art in micro/nano drug delivery systems within clinical settings and evaluate the impediments faced in translating these innovations from laboratory settings to practical bedside applications. Finally, we offer insights into the emerging micro/nanorobotic brain drug delivery systems, characterized by targeted transport, real-time image guidance, and closed-loop control mechanisms.

Prevalence of brain diseases

Brain and nervous system cancers constitute approximately 1%–2% of the total global cancer cases reported annually.⁷ Within this category, malignant brain

tumors, notably glioblastoma, stand out for their exceptionally high mortality rates and significant contribution to overall cancer-related fatalities. The 5-year relative survival rate for these tumors hovers at a meager 30%,⁸ and glioblastoma itself offers a particularly grim prognosis, with a median survival period of less than 2 years.⁹ Moreover, approximately 20% of cancer patients encounter brain metastases, which represent the primary cause of death in individuals afflicted by advanced cancer.¹⁰ Neurodegenerative diseases encompass a cluster of disorders characterized by the progressive degeneration of nerve cells, leading to declining motor function, memory, and cognition. Common neurodegenerative diseases include AD, PD, Huntington's disease, and ALS. Globally, an estimated 50 million individuals live with dementia, with higher prevalence rates observed in low- and middle-income countries, thus imposing a substantial burden on affected families and society at large.¹¹ Although other neurodegenerative diseases may exhibit lower incidence rates, their impact remains substantial.^{12–14} Stroke, including both ischemic and hemorrhagic strokes, stands as a significant contributor to disability and mortality worldwide, impacting one in four people during their lifetime, yet with limited treatment options.^{15,16} The cornerstone for the acute management of stroke presently resides in intravenous tissue plasminogen activator for thrombolysis.¹⁷ However, this therapy requires high doses, making it susceptible to complications and therefore demands controlled release mechanisms. Traumatic brain injury represents a grave form of brain damage occurring in over 20 million cases worldwide each year.¹⁸ All of these brain disorders share a common challenge: the intricacies of drug delivery to the brain, and excellent therapies are desperately needed.

Challenges of drug delivery to the brain

Drug development in the central nervous system (CNS) is well recognized as a challenging endeavor. Historical data indicate that approval rates by US Food and Drug Administration (FDA) for CNS drugs are less than half of those for non-CNS drugs, with prolonged approval timelines.⁴ These endeavors encounter several limitations including biological barrier penetration, cell-specific targeting, limited drug distribution, drug toxicity, and preclinical models.

The brain is safeguarded by a complex and intricately regulated biological barrier that serves the dual purpose of preventing the entry of harmful substances into the brain and controlling the delivery of drugs, as illustrated in Figure 1. These barriers primarily consist of the blood-brain barrier (BBB), blood-cerebrospinal fluid (CSF) barrier, and the arachnoid barrier.¹⁹ The BBB, in particular, constitutes a highly sophisticated structure that acts as a critical separation between the circulating blood and the interstitial fluid at the brain capillary site, thereby shielding the brain from potentially harmful substances. Positioned at the core of the neurovascular unit (NVU), the BBB is composed of brain microvessel endothelial cells (BMVECs) and the extracellular matrix (ECM). BMVECs are interconnected by tight junctions, resulting in low paracellular permeability, and express transporters and receptors that regulate the transcytosis of essential substance.²⁰ Pericytes are found in the perivascular region, maintaining the integrity and functionality of the BBB. The endfoot of astrocytes encircle the vessels, offering nutrients and facilitating the formation of tight junctions. The abluminal surface of endothelial cells (ECs) is coated by the basement membrane. Together, these components constitute the rest of the NVU.^{20–23}

CSF is a transparent, colorless fluid that envelops the brain and spinal cord and serves to cushion and provide nutrients while facilitating waste product excretion.²⁴ CSF is primarily generated by the choroid plexus in the lateral ventricles and the third and fourth ventricles, and flows via the fourth ventricle into the sub-arachnoid space of the brain and spinal cord, eventually being absorbed through the arachnoid villi into the superior sagittal sinus of the peripheral blood flow.²⁵ The epithelial cells of the choroid plexus establish the blood-cerebrospinal fluid barrier (BCSFB) that separates the CSF from the blood circulation. Unlike the BBB, the BCSFB exhibits higher permeability. Consequently, it is possible to

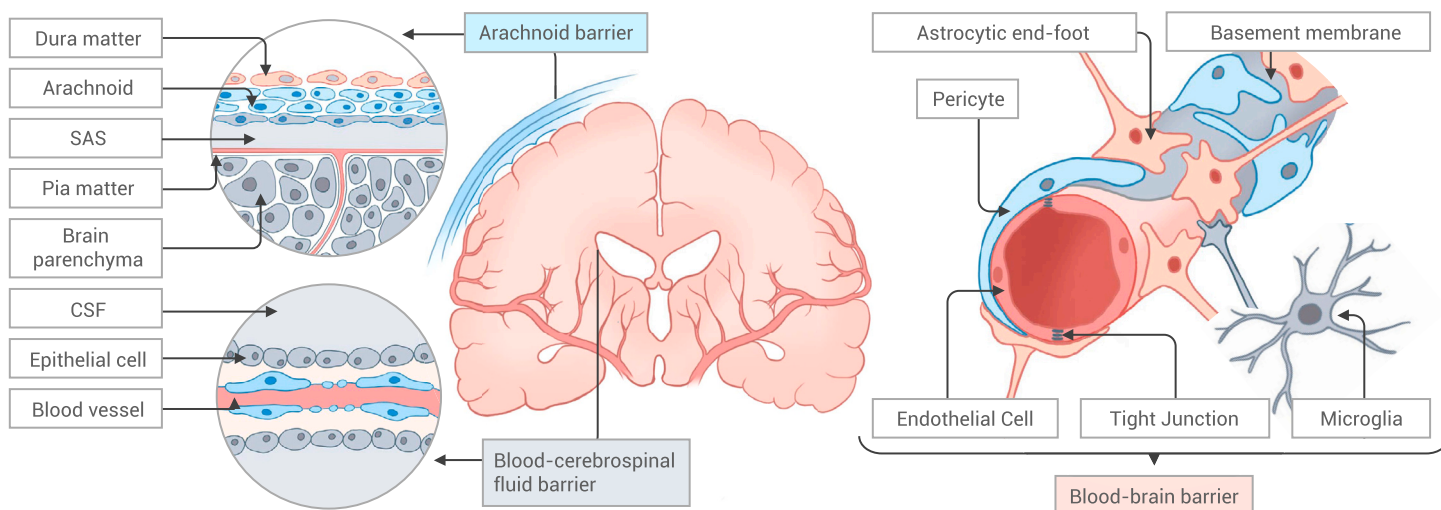


Figure 1. Biological barrier in the brain Biological barriers in the brain include the arachnoid barrier, the blood-cerebrospinal fluid barrier, and the blood-brain barrier. The blood-brain barrier consists of endothelial cells, pericytes, astrocyte endfeet, and basement membranes, distinguished by tight junctions between endothelial cells.

introduce drugs into the CSF, which can subsequently traverse into the bloodstream and, eventually, access the brain parenchyma via the BBB. However, this approach suffers from a low penetration rate. Alternatively, drugs can diffuse into the brain parenchyma through the CSF, but this typically necessitates high doses and is associated with the risk of inducing neurotoxicity.²⁵ Intrathecal and intracerebroventricular (i.c.v.) injections in nanomedicine hold new promise for CSF delivery, but still require meticulous control.²⁷ The arachnoid barrier, a distinctive type of BCSFB, consists of connective tissue and arachnoid cells that enshroud around the spinal cord and brain. The arachnoid barrier separates the fenestrated vessels of the dura mater from the CSF in the subarachnoid space, regulating the exchange of molecules into and out of the CSF. Recent studies have identified the developmental processes and crucial protective functions of the arachnoid barrier in prenatal CNS,²⁸ revealing how fetal barrier hypoplasia can render susceptibility to meningitis. Subarachnoid injections have been employed for anesthesia and analgesia and hold potential for other drug delivery purposes.²⁹

The scarcity of preclinical models capable of faithfully replicating the intricate structure of the human brain poses constraints on drug development and validation. Conventional *in vitro* models that emulate the BBB such as parallel artificial membrane permeability assays and transwell assays are structurally rudimentary and lack physiological fidelity.³⁰ *In vivo* experimental animal models also fall short in faithfully mirroring human biology due to interspecies variability.³¹ Recent developments in microfluidics have ushered in a novel approach for BBB research, namely BBB-on-chip models (μ BBBs). μ BBBs facilitate the co-cultivation of diverse human cell types and replicates essential physical processes, including shear stress, thus creating a meticulously controlled microphysiological system.³² The μ BBB platform is versatile, accommodating applications in the study of CNS pathology, high-throughput drug screening, and neurotoxicity assessments.^{33–35} When combined with organoids, it offers a more realistic emulation of the human brain and is poised to become the next-generation platform for CNS drug screening.³⁶

MICROFLUIDIC SYSTEMS FOR *IN VITRO* MODELS

Recent advancements in microfluidics and microfabrication technologies have facilitated the emergence of the organ-on-chip model for *in vitro* drug screening.³⁷ In the realm of brain research, the introduction of the μ BBBs has enabled the emulation of intricate human brain structures *in vitro*. The μ BBB typically comprises three-dimensional (3D) vascular endothelium formed by BMVECs and can be co-cultured with astrocytes and pericytes to more accurately replicate NVU structures.³⁸ The continuous perfusion provides shear stress to the vascular endothelium restoring the mechanical conditions the ECs are subjected to. The μ BBBs can be categorized into two main types based on cell distribution and cellular assembly: artificial structures and self-forming structures (Figure 2). In the self-forming model, BMVECs, astrocytes, and pericytes are co-cultured within an ECM gel, allowing them to autonomously develop into a 3D vascular network that closely resembles the native vascular architec-

ture.³⁹ Personalized medicine is made possible through the utilization of patient-derived induced pluripotent stem cell (iPSC)-derived brain microvascular endothelial cells (BMVECs) in constructing self-forming models.⁴⁰ Nevertheless, this model demands precise control over vascular EC patterning to enhance the efficiency of drug screening.

Artificial structures

The artificial structure of μ BBBs can be categorized into vertical structure, parallel structure, and tubular structure (Figure 3). The vertical design typically comprises two channels, top and bottom, separated by a porous membrane. The top channel represents the blood side and is typically composed of BMVECs or iBMVECs, while the bottom channel represents the brain side and generally includes astrocytes and pericytes.³⁸ These two channels create a conventional sandwich-like configuration that can be arranged either in an upward or downward orientation.^{40,41} The vertical design offers a substantial contact area, facilitating the examination of the mechanisms underlying the transport of substances across the BBB and their permeation effects. However, due to the overlapping nature of the upper and lower channels and the limited transparency of the porous membrane, obtaining high-resolution images with an inverted microscope can be challenging, necessitating the utilization of more complex 3D high-resolution imaging techniques.⁴⁷ Ahn et al. have effectively employed tri-culture vertical μ BBB systems to precisely observe the distribution and uptake of nanoparticles (NPs) within various cells.⁴¹ In addition, the simple structure of the vertical design allows for batch manufacturing for high-throughput drug screening.³⁴

The parallel design typically comprises multiple side-by-side channels interconnected by microchannels or micropillars. These narrow channels are capable of housing a single cell, thereby serving as interfaces for cellular communication without permitting cell migration across the channels. Microchannels offer the advantage of observing various processes, such as NP permeation, synaptic connections between neurons, and the infiltration of tumor metastatic cells at the single-cell level.^{48,54–56} Fan et al. developed a microchannels design to investigate the interaction of drug nanocarrier with BBB and revealed that Tf-PEG-AuNP exhibit exceptional BBB penetration with minimal protein absorption.⁴⁸ Constructing microfluidic models to explore interactions between neuro-glial cells in brain diseases enhances our comprehension of the underlying molecular mechanisms of pathogenesis and facilitates the screening of potential therapeutic approaches.^{57,58} To more faithfully replicate the human BBB structure, micropillar designs were developed. These designs are often used in combination with matrix gels on the brain side to emulate the basement membrane to which BMVECs adhere.⁴³ These micropillars typically possess a trapezoidal structure, with multiple micropillars arranged to generate surface tension and confine the hydrogel within the brain channel.⁵⁹ Shin et al. designed a multichannel AD BBB dysfunction *in vitro* model separated by multiple sets of micropillars to demonstrate the entry of neurotoxin thrombin into the brain through a compromised BBB, as well as to assess the potential mitigating effects of BBB enhancers on AD.⁴⁹

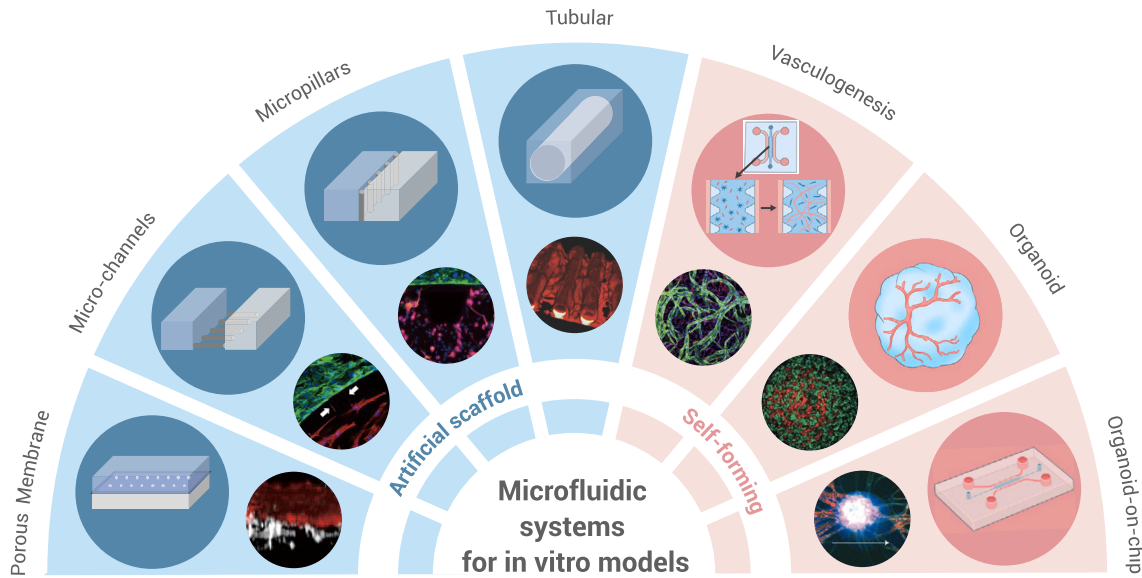


Figure 2. Microfluidic systems for *in vitro* models Microfluidic systems offer the capability to create both artificial and self-forming *in vitro* models. Artificial scaffold designs primarily encompass porous membranes, microchannels, micropillars, and tubular configurations. Meanwhile, self-forming *in vitro* models predominantly include vasculogenesis designs, organoids, and organoids-on-chip. Microscopic images were reproduced from Campisi and co-workers.^{39,41–46} Copyright 2020, Springer Nature; 2019, Wiley Periodicals; 2021, Springer Nature; 2018, Wiley-VCH; 2018, Elsevier; 2013, Springer Nature; 2017, The Royal Society of Chemistry.

The above two μ BBB structures are typically fabricated by mask-based photolithography resulting in rectangular cross-sectional channels. However, disparities in the morphology of the EC layer emerge between the cuboid geometry and the vessel-like cylindrical structure due to variations in shear stress distribution.³⁰ Consequently, a novel fabrication method has been introduced to produce full-contact tubular μ BBB designs, which more faithfully replicate the mechanical properties of *in vivo* blood flow to ECs. One approach involves the pre-embedding of cylindrical microneedles into polydimethylsiloxane, followed by their removal after curing, to establish microchannels.^{50,60,61} For example, Seo et al. constructed 3D vascular structures and co-cultured them with glioma spheres to validate combination therapy with BBB-opening and chemotherapeutic agents.⁵⁰ Furthermore, two-photon polymerization (TPP)-based stereolithography permits the fabrication of intricate microstructures for constructing tubular vascular scaffolds.⁶² Ciofani and co-workers constructed both two-component and three-component μ BBB models by printing side-by-side tubular structures with microporosity in a single molding process using TPP technology.^{44,63} The demonstrated utility of TPP for microcomponent processing in microfluidic chips carries significant implications for μ BBB applications.⁶⁴ The above three artificial μ BBB structures realize regular and controllable cellular arrangements, which have great applications in the fields of CNS drug screening and brain pathological study.

Self-forming structures

The artificial μ BBB structure aims to create engineered microvessels within a designated framework. Yet, there remains a gap between these structured vessels and the intricate capillary network of the human body. In recent years, self-forming μ BBB structures have emerged, including the vasculogenesis design and vascularized organoids on a chip, which enable the formation of 3D brain microvascular networks (Figure 3).^{32,36} The vasculogenesis design employs iPSC-derived ECs or primary BMVECs to generate irregular vascular networks and are coupled with primary pericytes and astrocytes to form BBB structures.^{39,65} Human pluripotent stem cells can also derive pericytes that can be integrated into the models described above.⁶⁶ Kamm and co-workers used this system to construct a 3D self-organized microvascular model and obtained gene expression profiles and quantitative immunocytochemistry similar to that of natural BBB and with lower permeability than conventional μ BBBs.^{32,39} Moreover, some researchers have developed the angiogenesis design for μ BBB models to simulate the angiogenic sprouting during BBB formation in a microfluidic context.^{67,68} This design is useful for investigating various aspects of BBB generation, such as the role of various perivascular cells,⁶⁹ the role of interstitial flow,⁷⁰ and the mechanisms of BBB regeneration.⁵¹ Kim et al. demonstrated the potential of stem cell therapy in BBB injury regeneration using an angiogenesis

microfluidic chip.⁵¹ To achieve a predetermined vascular distribution, ECs can be patterned within hydrogels. Several pre-patterning methods based on geometric constraints,⁷¹ 3D printing,⁷² and temporary scaffolds⁷³ have been employed to generate regular vessels. Acoustophoresis, as a non-contact cell manipulation method, also holds potential for controlled vasculogenesis.⁷⁴

The hydrogel-based self-forming vascular model described above exhibits a limitation in its interaction with NVU cells, thereby diverging from the *in vivo* context. To address this issue, 3D cellular aggregates, such as spheroids and organoids, composed of multiple organ-specific cells, offer a solution by facilitating optimal intercellular contact.⁷⁵ The spheroid μ BBB model is structured by coating primary or immortalized astrocytes and pericytes on spheroids formed by BMVECs.⁵² A notable protocol devised by Cho and co-workers successfully generated BBB spheroids comprising ECs, astrocytes, and pericytes. This advancement enabled effective screening of CNS therapeutics through optical and mass spectrometry techniques.^{52,76} Furthermore, the incorporation of various NVU cell types, including neurons, oligodendrocytes, and microglia, into these spheroids has improved their ability to mimic drug penetration within the body.^{77,78} Distinct from spheroids, the organoid μ BBB model represents a cellular aggregate generated from stem cells.⁷⁹ Cerebral organoids generated from human embryonic stem cells or human iPSCs have become well established.⁸⁰ However, vascularization of cerebral organoids has only recently been developed to address the limitations in CNS drug screening.^{81–83} It is worth noting that constructing a BBB organoid typically spans several months and presents a complex internal structure, necessitating additional efforts to translate it into a drug screening model.

Recent techniques have emerged for integrating 3D cellular aggregates into microfluidic chips, creating "organoid-on-chip" systems. Ao et al. developed a microfluidic chip for dynamic perfusion of human brain organoids to study immune cell-driven brain aging.⁸⁴ Microfluidic chips also offer high-throughput capabilities,⁸⁵ demonstrated by Eilenberger et al., who created spheroid arrays on these chips to assess BBB permeability.⁵³ Recently, micro-electro-mechanical systems have also been applied to brain organoid-on-chip. Huang et al. fabricated shell microelectrode arrays to record spatiotemporal signals from brain organoids.⁸⁶ In conclusion, self-forming μ BBBs aim to faithfully mimic the human BBB, offering potential for improved CNS therapeutics in preclinical trials.

In the broader context, artificial and self-forming structures exhibit distinctions in their physiological relevance and processing convenience, rendering them suitable for distinct objectives. Artificial structures offer advantages by providing well-defined and stable culture conditions.⁸⁷ Moreover, they offer a clear optical window enabling high-resolution imaging.³⁰ Consequently, artificial structures find utility in commercial settings for applications like brain drug screening, neurotoxicity testing, and personalized medicine due to their controllability and

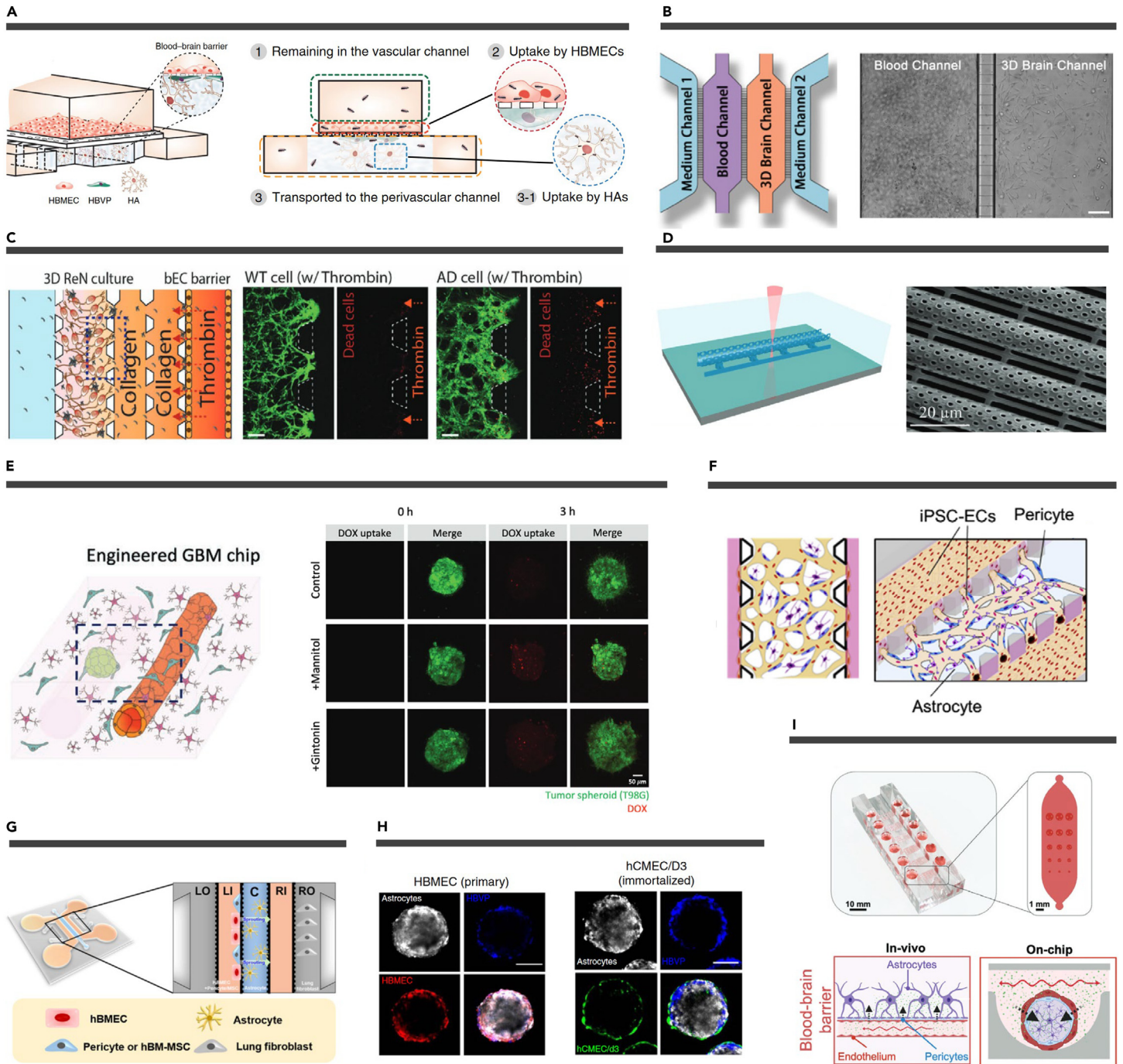


Figure 3. Artificial structures and self-forming structures of μ BBBs (A) Schematic of a vertically designed μ BBBs containing three types of cells. Reproduced from Ahn et al.⁴¹ Copyright 2020, Springer Nature. (B) Schematic diagram (left) and bright-field images (right) of a microchannel-profiled μ BBB model. Scale bars, 100 μ m. Reproduced from Fan et al.⁴⁸ Copyright 2023, The Royal Society of Chemistry. (C) A micropillars-designed μ BBB to mimic damage to nerve cells by thrombin influx through a damaged BBB. Dead cells are shown in red. Scale bars, 200 μ m. Reproduced from Shin et al.⁴⁹ Copyright 2019, Wiley-VCH. (D) Fabrication schematic (left) and SEM image (right) of a TPP printed tubular μ BBB design. Reproduced from Marino et al.⁴⁴ Copyright 2018, Wiley-VCH. (E) Schematic of a tubular design μ BBB + GBM (left). Fluorescent images showing the uptake of DOX by GBM spheres (right). Reproduced from Seo et al.⁵⁰ Copyright 2022, Wiley-VCH. (F) Schematic representation of a vasculogenesis BBB microvascular network model that mimics the microvascular structure in the brain environment. Reproduced from Campisi et al.³⁹ Copyright 2018, Elsevier. (G) Schematic diagram of the angiogenesis microfluidic chip. Reproduced from Kim et al.⁵¹ Copyright 2021, Elsevier. (H) Representative confocal images showing the organization of human astrocytes (white), HBVP (blue), and (left) primary HBMEC (red) or (right) immortalized human cerebral microvascular ECs (hCMEC/D3; green) when co-cultured to form a spheroid. Reproduced from Cho et al.⁵² Copyright 2017, Springer Nature. (I) A cutaway rendering of the microfluidic spheroid array (top). Overview of the established 3D BBB model system (bottom). The arrows indicate the transport of the drug. Reproduced from Eilenberger et al.⁵³ Copyright 2021, Wiley-VCH.

standardized modeling. Conversely, self-forming structures excel in physiological relevance and microenvironment control, closely resembling the natural microenvironment of organs and tissues.³⁰ While the setup for self-forming structures is increasingly standardized, its technical demands restrict large-scale applications. Self-forming structures are better suited for research-focused objectives such as brain tumors, neuroinflammatory and neurodegenerative diseases, viral infections, and neurodevelopment due to their high fidelity to human physiology.

In the context of *in vitro* BBB models, both artificial and self-forming structures must prioritize integrity and validity concerning their resemblance to the human BBB. The integrity of μ BBBs can be assessed through measurements of trans-epithelial/transendothelial electrical resistance (TEER) and the permeation of fluorescent dyes.^{32,88} Currently, μ BBBs featuring human iPSC-derived ECs co-cultured with astrocytes can achieve TEER values of 4,000–5,000 Ω cm^2 ,⁸⁹ closely approximating the *in vivo* value of 5,900 Ω cm^2 .⁹⁰ To further test integrity,

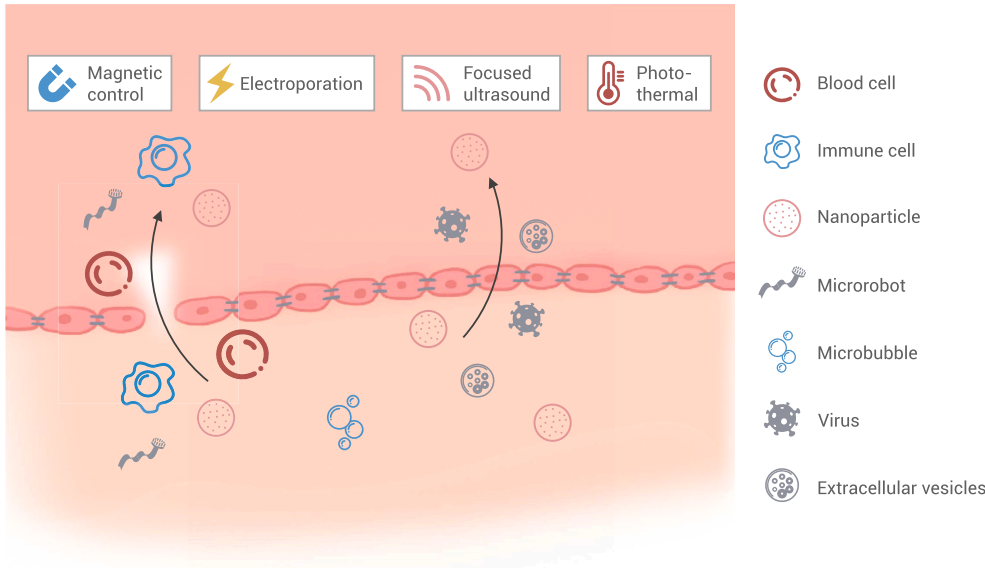


Figure 4. Micro/nanomatters for vasculature-brain drug delivery Both biological carriers and artificial micro/nanomatters can cross the BBB through intercellular and intracellular routes. The BBB, along with micro/nano materials, can be controlled using external stimuli, such as magnetic fields, electroporation, focused ultrasound, and photothermal.

permeability experiments utilizing small-molecule fluorescent probes, including fluorescently labeled dextrans of varying molecular weights, are conducted.⁹¹ To establish the validity of *in vitro* BBB models, these models must replicate key attributes of the natural BBB. Expression of tight junction proteins ZO-1 and claudin 5, adherens junction proteins CD31 and VE-cadherin, basement membrane proteins laminin and collagen IV, glycoalyx proteins heparan sulfate proteoglycan 2, and hyaluronic acid should be confirmed in μ BBBs by immunofluorescence staining.³² Furthermore, the gene expression profiles related to the BBB should align with *in vivo* profiles, confirmed through RT-qPCR.³⁹ *In vitro* BBB models can also be assessed for the secretion of cytokines affecting BBB function, such as C-C motif chemokine ligand 2 (CCL2), IL-8, and IL-6, using ELISA.⁹² Finally, direct comparisons between *in vitro* BBB models and *in vivo* BBB in mice, demonstrating consistent permeability, are now achievable through intravital microscopy.⁹³








Micro/nanomatters for vasculature-brain drug delivery

The application of micro/nanosystems in brain drug delivery is primarily categorized into two approaches: systemic delivery and interventional delivery.⁹⁴ Systemic administration primarily involves vasculature-brain drug delivery, typically through intravenous means. BBB blocks over 98% of small-molecule drugs and nearly 100% of large-molecule drugs.⁹⁵ Micro/nanomatters provide three primary strategies to surmount this challenge: (1) employing carriers of biological origin, (2) engineering carriers with the ability to penetrate the BBB, and (3) regulating the transient opening of the BBB (Figure 4).

Biological carriers

Biological carriers have been engineered to function as innate vehicles for drug delivery due to their intrinsic capacity to target and cross the BBB. Examples of such carriers include blood cells,⁹⁶ neurotropic viruses,⁹⁷ and extracellular vesicles (EVs).^{98,99} In addition, cell-based microrobots have been designed for this purpose.¹⁰⁰ The utilization of biological carriers presents a compelling approach to drug delivery (Figure 5).

Cell-based delivery. The application of biological cells as drug carriers falls within the realm of cell therapy.¹⁰⁷ Various immune cell types such as neutrophils,¹⁰¹ monocytes,¹⁰⁸ macrophages,¹⁰⁹ dendritic cells,¹¹⁰ and Th17 cells¹¹¹ have been employed for targeted brain drug delivery. These immune cells are recruited to brain tumors, penetrating glioma sites and continuing to be recruited through the release of inflammatory factors post-resection. To illustrate, Xue et al. loaded liposomes containing paclitaxel into neutrophils, effectively inhibiting glioma recurrence following surgery.¹⁰¹ Dendritic cells can also enhance immunosuppression against tumors and deliver chemotherapy drugs.¹¹⁰ Red blood cells, being abundant in the bloodstream, can be modified for brain drug delivery.¹¹² Recent studies have explored using hematopoietic stem cells to treat brain metastases more effectively.^{113,114} Nevertheless, cell-based drug delivery is still emerging and faces challenges in terms of cell localization within the brain, controlled navigation, and potential systemic toxicity. For example, Zhang et al.

-  Blood cell
-  Immune cell
-  Nanoparticle
-  Microrobot
-  Microbubble
-  Virus
-  Extracellular vesicles

engineered "neurobots" by encapsulating drug-loaded magnetic nanogels in *E. coli* membranes within neutrophils. These neurobots demonstrated navigation and enrichment in the vasculature under the influence of an external magnetic field, crossing the BBB using chemotactic movement driven by inflammatory factors, and inhibiting tumor cell proliferation.¹⁰⁰ However, further refinement is needed to optimize their therapeutic efficacy and spatiotemporal control before they can become promising drug delivery vectors.

Neurotropic viruses. Neurotropic viruses, represented by adeno-associated viruses (AAVs), have become significant in neurological gene therapy due to their ability to target various brain cells.^{97,115} In clinical practice, intraparenchymal injection is common but invasive and limited in coverage.^{116,117} Although subtypes of AAV, particularly AAV9, have shown the ability to transduce neurons and glial cells via intravenous injection, they are hampered by low efficiency and require high doses.^{118,119} To address this, researchers have developed advanced AAV variants with improved tropism and BBB penetration through rational design and screening of the AAV capsid.¹²⁰ Gradinaru and co-workers evolved AAV-PHP.B and AAV-PHP.eB, achieving 40-fold higher CNS transduction in mice.^{121,122} They further created AAV.CAP-B10 with low hepatotoxicity, successfully expressing genes across the brain in marmosets.¹²³ Yao et al. incorporated cell-penetrating peptides into AAV9, leading to AAV.CPP.16 and AAV.CPP.21, with 5-fold higher CNS transduction in cynomolgus macaques.¹⁰² These effective AAV toolkits provide promising opportunities for systemic gene therapy for a wide range of CNS diseases.

EVs. EVs, comprising exosomes, microvesicles, and apoptotic bodies, are cell-released membrane-derived vesicles that facilitate intercellular communication and bioregulation.¹²⁴ EVs have gained widespread recognition for their clinical diagnostic and therapeutic potential, and they are actively under investigation as innovative drug delivery agents. Key intrinsic features of EVs include their size, which ensures stability in circulation, their endogenous composition, allowing immune system evasion, and, notably, the capacity of certain cell-derived EVs to cross the BBB.¹⁰⁷ These attributes make EVs efficient drug delivery vehicles for brain diseases.⁹⁸ EVs can be loaded with various cargoes including exogenous nucleic acids (miRNA, short interfering RNA [siRNA], mRNA) and proteins.¹²⁵ EVs from specific sources (e.g., macrophages,¹²⁶ tumor cells,¹²⁷ neutrophils,¹²⁸ and choroid plexus¹²⁹) can cross the BBB or BCSFB without the need for targeted modification. Cui et al. extracted exosomes from serum, loaded them with the chemotherapeutic drug nanomicelles, and anchored the Toll-like receptor 9 agonist CpG oligonucleotide to the exosome membrane creating CpG-EXO/TGM.¹⁰⁴ Surface engineering of EVs can enhance their brain-targeting abilities.¹³⁰ Genetic methods are employed for surface modification, where plasmids expressing a targeting peptide (e.g., rabies virus glycoprotein [RVG]) are transduced into endogenous cells, and EVs with the targeting peptide (e.g., RVG-Lamp2b fusion protein) on their surface are subsequently purified.¹³¹ Alternatively, targeted proteins can be directly attached to EV membranes using click chemistry. Peptides like c(RGDyK) or stromal cell-derived factor 1 have been conjugated to EV membranes using click chemistry for the treatment of cerebral ischemia.^{132,133} However, challenges like low purification and loading efficiency need resolution for EVs to become effective drug carriers.¹⁰⁷

Artificial micro/nanomatters

The development of micro/nano technology in the past decade has led to the creation of artificial micro/nanomatters for drug carriers, including nanoparticulate systems, micro- and nanorobots, and microbubbles (MBs) (Figure 5). These

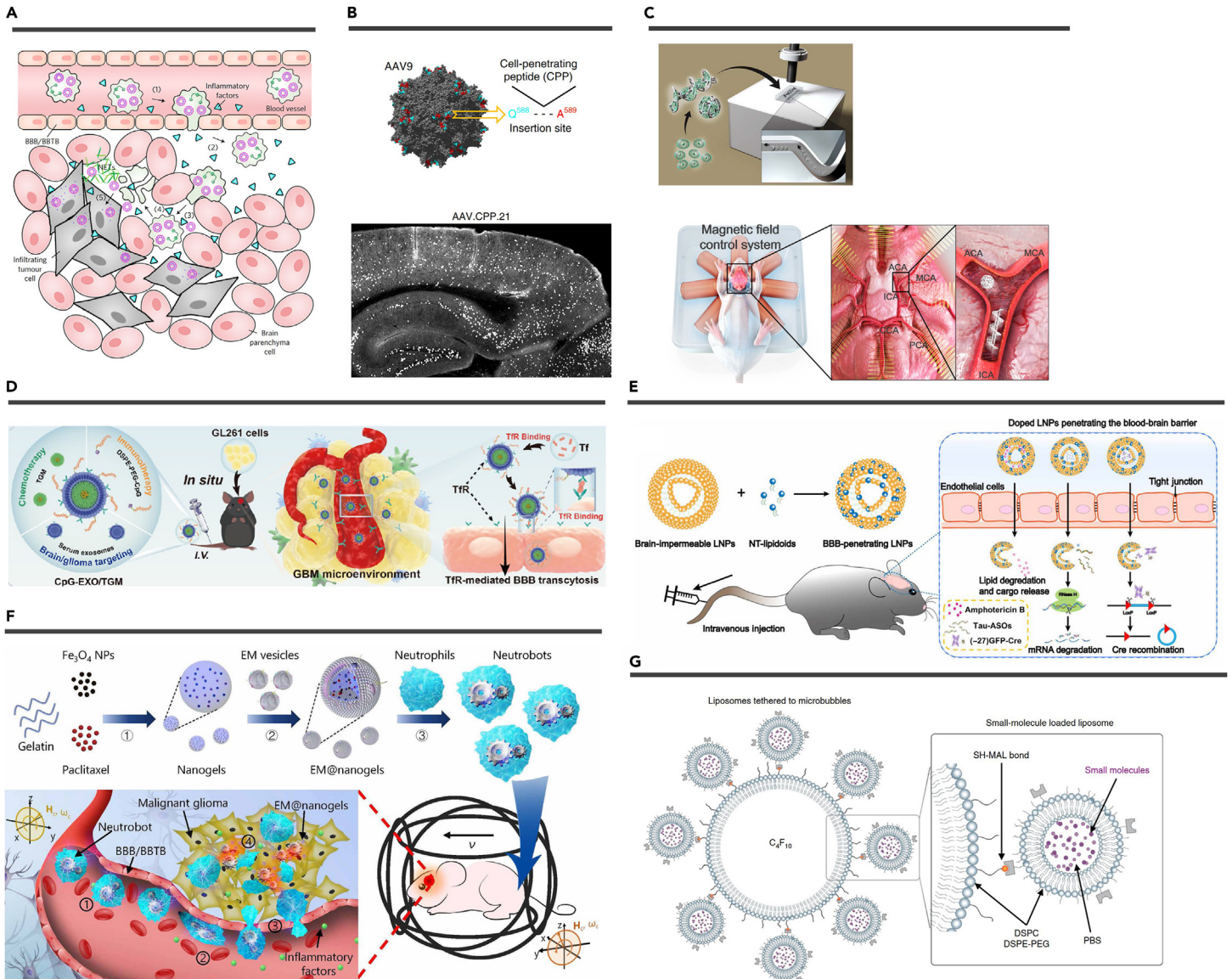


Figure 5. Artificial micro/nanomatters (A) Schematic that shows how engineered neutrophil target glioma after intravenous injection into mice. Reproduced from Xue et al.¹⁰¹ Copyright 2017, Springer Nature. (B) AAV9 capsid model showing the insertion site for CPPs (top). Representative images of mouse brain regions showing successfully transfected cells by AAV.CPP.21 (white dots) (bottom). Reproduced from Yao et al.¹⁰² Copyright 2022, Springer Nature. (C) Schematic of the microrobots (top). *Ex vivo* model of a brain blood vessel and the magnetic field control system (bottom). Reproduced from Jeon et al.¹⁰³ Copyright 2019, American Association for the Advancement of Science. (D) After CpG-EXO/TGM enters the blood circulation, the TfR on its surface can combine with free Tf in the blood, so that CpG-EXO/TGM is endowed with the ability to target BBB and GBM cells. Reproduced from Cui et al.¹⁰⁴ Copyright 2023, American Chemical Society. (E) Schematic illustration of formulating NT-lipidoid-doped LNPs for cargo delivery into the brain. Reproduced from Ma et al.¹⁰⁵ Copyright 2020, American Association for the Advancement of Science. (F) Schematic of active therapeutics of dual-responsive neurobots *in vivo*. Reproduced from Zhang et al.¹⁰⁶ Copyright 2021, American Association for the Advancement of Science. (G) Schematic representation of small-molecule-loaded liposomes tethered to microbubbles. Reproduced from Ozdas et al.¹⁰⁶ Copyright 2020, Springer Nature.

artificial micro/nanostructures possess targeting capabilities and can cross the BBB, making systemic administration an effective therapeutic option. They address limitations of poor stability of free drugs, rapid enzymatic degradation, inadequate release, and poor pharmacokinetics.^{19,134} While relatively few micro/nanomatters are currently approved for treating CNS diseases, much exploration is underway.

Nanoparticulate systems. NPs utilized for systemic brain drug delivery primarily comprise lipid-based NPs, polymeric NPs, and inorganic NPs. Lipid-based NPs, such as liposomes and lipid NPs, are non-toxic carriers with phospholipid mono- or bilayers and have high cargo loading efficiency.¹³⁵ These NPs are typically constructed from biodegradable materials with hydrophilic heads and hydrophobic tails, including cholesterol, phospholipids, and fatty acids.¹³⁶ Clinical trials involving liposome-loaded chemotherapeutic agents (e.g., doxorubicin, irinotecan) for glioma treatment are already underway.^{137–139} Liposomes have also emerged as vehicles for gene delivery, most notably in the clinical application of lipid NP-mRNA vaccines against COVID-19, marking a milestone in mRNA therapeutics.¹⁴⁰ Lipid-based NPs efficiently transport nucleic acids to

the brain for addressing neurodegenerative diseases, brain tumors, and brain inflammation.^{141–144} Ma et al. introduced neurotransmitter-derived lipidoids into liposomes for BBB crossing.¹⁰⁵ This enabled the successful delivery of antibiotics, antisense oligonucleotides, or Cre recombinase to the mouse brain through systemic intravenous administration, achieving gene silencing or recombination in neuronal cells.¹⁰⁵

Polymeric NPs created from homopolymers or copolymers are a promising method for drug delivery to the brain. Common polymeric NPs encompass micelles, nanocapsules, nanospheres, polymersomes, and more.¹⁴⁵ These polymeric NPs, derived from biogenic monomers or featuring surface biomodifications, offer outstanding biocompatibility and biodegradability, making them valuable in diverse therapeutic applications for brain diseases. For example, Wu et al. used biocompatible polydopamine and conductive polymer polypyrrole to create NPs loaded with the antiepileptic drug phenytoin, enabling BBB crossing and controlled drug delivery.¹⁴⁶ Poly(lactide-co-glycolide) acid, the most successful US FDA-approved biomedical polymer,¹⁴⁷ has seen rapid development in polymeric NP-based treatments for neurodegenerative

diseases.¹⁴⁸ In addition, polymeric NPs have been explored for various therapies in the context of brain tumors. CRISPR-Cas9 nanocapsules, synthesized from crosslinked polymers with disulfide bonds, have been employed for gene therapy in glioblastoma.¹⁴⁹ NPs loaded with siRNA, synthesized from polymerized human serum albumin and oligo(ethylene glycol), have been used for a similar purpose.¹⁵⁰ Furthermore, nitric oxide-propelled chemotactic nanomotors, synthesized from L-arginine derivatives as monomers, have enhanced immunotherapy in the context of glioblastoma.¹⁵¹

Inorganic NPs, with unique properties like optical, electrical, and magnetic characteristics, are promising for diagnosing and treating brain diseases.¹⁵² Gold nanoparticle (AuNP) is widely employed in photothermal therapy (PTT) and theranostics for cancer because of its high photothermal conversion efficiency and good photostability.¹⁵³ Importantly, AuNPs can cross the BBB via passive diffusion and endocytosis without compromising BBB integrity.^{154,155} Silica NPs, especially mesoporous silica nanoparticles (MSNs), offer superior chemical stability compared with polymeric NPs.¹⁵⁶ MSNs possess a high specific surface area, facilitating effective drug loading.^{157,158} Tao et al. synthesized MSNs loaded with arsenic trioxide, demonstrating a 2.34-fold increase in pharmacokinetics when targeting brain gliomas compared with a solution.¹⁵⁹ Magnetic NPs (MNPs) enable precise manipulation for brain localization, which is discussed in the next section.¹⁶⁰ While NP-based brain drug delivery systems hold clinical potential, their BBB penetration and brain targeting require further validation. μ BBB models aid in NP infiltration studies,¹⁶¹ and ensuring safety and stability remains a challenge.

Micro- and nanorobots. Micro- and nanorobots are miniature actuators operating at micro- and nanoscales. They have demonstrated considerable potential in biomedicine due to their ability to convert various energy sources into motion and force.¹⁶² This rapidly evolving field of robotics research has made notable progress in diverse applications such as drug and cell delivery, microsurgery, cell characterization, pathogen sensing, biopsy, and medical imaging.^{163,164} Their unique capability to access tight cavities, typically beyond reach by conventional means, enhances treatment efficacy.¹⁶⁵ These robots are categorized by actuation methods, encompassing chemical, external stimuli (e.g., optical, ultrasonic, electrical, magnetic), and bio-hybrid actuation.¹⁶² Through active propulsion or manipulation via external fields, micro- and nanorobots exhibit precise mobility within brain vessels. Surface modifications enable BBB traversal, such as the development of glucose chemotaxis-driven nanoswimmers functionalized with angiopep-2 for BBB penetration.¹⁶⁶ Biocompatible micro- and nanorobots facilitate cell attachment and transport, showing promise in cell therapy and neurological injury repair. Jeon et al. utilized TPP for the fabrication of microrobots with diverse geometries, serving as a structural framework for neuronal cell cultivation. Neural stem cells adhered to these microrobots, subsequently proliferated, and underwent differentiation into astrocytes, oligodendrocytes, and neurons.¹⁰³ The precise manipulation of these microrobots within mouse brain slices and rat brain vessels was achieved through external magnetic field control.¹⁰³ The same research group created neuron-loaded microrobots to selectively establish neural network connections for in-depth neural communication studies.¹⁶⁷ However, the micro- and nanorobotics field requires continued exploration in materials, fabrication, actuation, and intelligence. In addition, research should focus on swarm manipulation to harness swarm intelligence for more effective treatment.

MBs. Most drug delivery systems targeting the BBB rely on biologically endogenous mechanisms that do not disrupt its integrity. Conventional methods for enhancing BBB penetration using chemical and biological stimuli lack precision and pose a high risk of toxicity.¹⁶⁸ In contrast, focused ultrasound (FUS) in conjunction with intravenous MBs can safely and temporarily open the BBB at specific sites through cavitation effects.¹⁶⁹ As early as 2001, Hynynen et al. combined MBs with FUS (FUS-MB) and used magnetic resonance imaging (MRI) for real-time monitoring, significantly reducing the required ultrasound power without harming surrounding neurons, resulting in MRI-guided focused ultrasound (MRgFUS).¹⁷⁰ US FDA-approved MBs for marketing, such as Lumason, Optison, and Definity, consist of a phospholipid or albumin shell and a perfluorocarbon or sulfur hexafluoride gas core,¹⁷¹ with diameters smaller (1–4 μ m) than red blood cells, facilitating passage through brain capillaries.¹⁷¹ Recent research investigates the impact of MB shape on biomedical applications. Non-spherical, anisotropic MBs were found to closely approach blood vessel walls and exhibit longer circulation times *in vivo*. Consequently, under the same ultrasound stimulation, non-spherical MBs exhibit higher BBB perme-

ability compared with spherical ones.¹⁷² MBs can be co-injected with therapeutic agents to enhance drug penetration under FUS. Proteins, AAV, NPs, siRNA, and chemotherapeutic agents have demonstrated improved penetration efficiency in mice when co-injected with MBs.^{173–178} Clinical trials have indicated that MRgFUS enhances the effectiveness of chemotherapeutic agents such as doxorubicin, temozolomide, and carboplatin in brain tumors while ensuring patient safety.^{179,180} However, due to differing pharmacokinetics and the potential for non-specific toxicity,¹⁷¹ drug-loaded therapeutic and theranostic MBs are also considered options.¹⁸¹ For instance, Liang et al. incorporated indocyanine green onto MB surfaces for real-time NIR-II fluorescence monitoring and PTT of glioblastoma,¹⁸² while Fan et al. developed folate-conjugated DNA-loaded MBs for enhanced targeted gene transfection efficiency in tumor cells expressing the folate receptor.¹⁸³ The temporary opening of the BBB allows blood components to enter the brain, which may lead to CNS toxicity, necessitating further verification of the safety of FUS-MB in humans. MBs can also be used to tether liposomes for precise drug aggregation and uncaging without opening the BBB under precisely modulated FUS, thus avoiding potential neurotoxicity.¹⁰⁶

Manipulation of the micro/nanomatters and BBB

Controlled drug delivery systems for the brain necessitate the precise manipulation of micro/nanomatters. The exact control of micro/nano therapeutic agents within the body primarily relies on the application of magnetic fields, acoustic fields, light fields, or electric fields. Moreover, achieving the reversible and secure opening of the BBB demands meticulous tuning of external stimuli. The principal modalities for BBB manipulation encompass FUS, photothermal effects, and pulsed electric fields (PEFs).¹⁹ In this section, we provide a comprehensive overview of several prominent methods for external manipulation (Figure 6).

Magnetic control. Magnetic fields provide a non-invasive, precise means for controlling MNPs and microrobots in drug delivery.^{19,187,188} Recent advancements in therapy and biosafety have expanded MNP clinical potential. For instance, Li et al. used MNPs in engineered siRNA-loaded exosomes to induce a novel cell death mode, ferroptosis, in glioblastoma cells.¹⁸⁹ Zhang et al. successfully manipulated MNP-loaded neutrophils at the cellular level.¹⁰⁰ Wang et al. employed intra-tumoral injection of magnetic carbon nanotubes, combined with precise magnetic field control, to induce rotational motion in these nanotubes, resulting in cancer cell death.¹⁹⁰ A diverse array of magnetic micro/nanorobots has been designed and applied in biomedicine, demonstrating substantial potential for clinical application.¹⁹¹ For instance, Wrede et al. recently introduced cell-sized spherical Janus magnetic microrobots, enabling real-time optoacoustic imaging and magnetic manipulation within the mouse brain vasculature.¹⁶⁴ This group also developed a wireless stent-shaped magnetic soft robot capable of shape-adaptive motion under magnetic field control, targeting the middle cerebral artery for thrombolysis and flow diversion, offering potential solutions for acute ischemic stroke and aneurysm management.¹⁹² Currently, there is a growing interest in swarm magnetic control for micro- and nanorobots, as it overcomes limitations associated with individual particles.¹⁹³ Wang et al. explored the use of microrobot swarm magnetism for active intravascular delivery across various blood flow environments.¹⁹⁴ In addition, they incorporated deep learning to enable microrobot swarms to autonomously adjust their movement and distribution in real-time based on their surroundings, enabling traversal through narrow and curved passages.¹⁹⁵ Wang et al. employed C-shaped magnetic fields to guide NP swarms for *in vivo* thrombolysis.¹⁹⁶ In another study, Zheng et al. fabricated a starfish-like shape-morphing microrobot capable of autonomously morphing to grasp and release numerous MNPs for swarm motion.¹⁹⁷

Ultrasonic control. FUS-mediated BBB opening, as a method to enhance brain drug delivery efficiency, has demonstrated safety and clinical viability based on preclinical trials.¹⁹⁸ Recent clinical trials have further confirmed its safety and efficacy in various neurological conditions, including glioma,^{179,199} brain metastases,²⁰⁰ AD,^{201,185} PD,^{202,203} and ALS.²⁰⁴ Ultrasound induces BBB opening through various physical effects within blood vessels, particularly mechanical effects.²⁰⁵ The combination of FUS with MBs results in significant mechanical effects, specifically cavitation effects, such as stable cavitation and inertial cavitation, linked to acoustic pressures.²⁰⁶ High acoustic pressure leads to the rapid collapse of MBs, generating high-speed microjets, shock waves, and sonoporation, which perforate the vascular EC membrane.²⁰⁷ However, strong inertial cavitation can lead to side effects, such as microvascular leakage and neuroinflammation, making it less practical.²⁰⁸ Therefore, using larger-diameter MBs (4–5

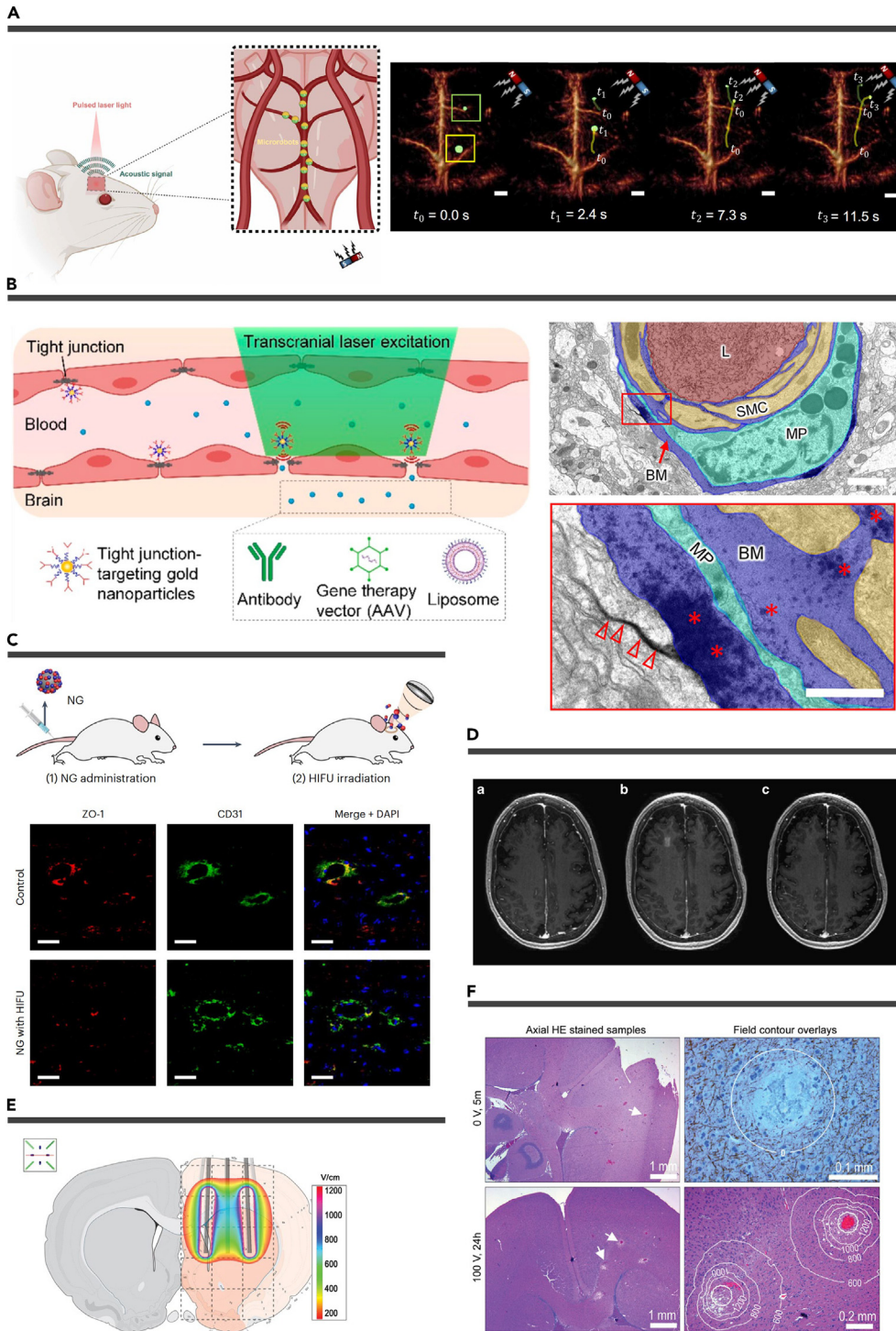


Figure 6. Manipulation of the micro/nanomatters and the BBB (A) Schematic representation of the non-invasive optoacoustic tomography of magnetic microrobots inside the murine brain vasculature. Reproduced from Wrede et al.¹⁶⁴ Copyright 2022, American Association for the Advancement of Science. (B) Schematic representation of reversible modulation of the BBB by laser stimulation of molecularly targeted nanoparticles (left). Electron microscopy imaging of lanthanum diffusion into the basement membrane (*) and interstitial space (empty arrowheads) (bottom). Reproduced from Li et al.¹⁵⁴ Copyright 2021, American Chemical Society. (C) Experimental schemes for nanoparticle administration and HIFU irradiation (top). Representative immunofluorescence images of midbrain sections co-stained for ZO-1 (red), CD31 (green), and nuclei (blue) (bottom). Scale bars, 30 μm . Reproduced from Kim et al.¹⁵⁴ Copyright 2023, Springer Nature. (D) MRI demonstration of BBB opening and closure. Axial T1-weighted gadolinium MRIs of patient 5 at (1) baseline, (2) immediately after stage 2 sonication and BBB opening, and (3) at 24 h after the procedure. Reproduced from Lipsman et al.¹⁸⁵ Copyright 2018, Springer Nature. (E) The electric field overlay (200 V) on the coronal plane corresponds to the center of the electrode device. (F) Rat brain histology after different voltage treatments. Reproduced from Mahmood et al.¹⁸⁶ Copyright 2015, Informa UK Limited.

are needed to validate its safety and optimize acoustic irradiation parameters and MB concentrations for different MBs. Moreover, FUS can be utilized for external stimulation of NPs, as demonstrated by Kim et al., who used FUS to activate piezoelectric NPs for deep brain stimulation and nitric oxide release to disrupt the BBB.¹⁸⁴

Other manipulation methods. Apart from the methods mentioned above, photothermal controls and PEFs can also be employed for physical BBB opening. NPs, when stimulated by near-infrared light, generate a thermal effect that can reversibly open the BBB.^{213–219} Li et al. synthesized AuNPs designed to target tight junctions, enhancing BBB permeability after transcranial laser stimulation, enabling the entry of antibodies, AAV, and liposomes.¹⁵⁴ Electromagnetic fields have demonstrated the ability to induce BBB opening and PEFs showing more promising results.^{220,221} Mahmood et al. conducted electroporation in the right hemisphere of rats using different voltages (100 and 400 V) and differentiated between transient and permanent membrane permeation using diffusion-weighted MRI.¹⁸⁶ However, these methods are less safe than ultrasound and have yet to find widespread clinical application.

and 6–8 μm) at lower pressure amplitudes (0.2–0.3 MPa) is considered a more promising clinical approach.²⁰⁷ At low acoustic pressure, MBs undergo periodic expansion and contraction, resulting in rubbing against the vessel wall and oscillation, generating microstreaming that exerts shear stress on the cell membrane.²⁰⁹ These effects lead to the upregulation of EC carrier proteins and receptor-mediated transcytosis, as well as the downregulation of tight junction proteins, facilitating drug passage through the BBB via trans- and paracellular pathways.²¹⁰ In addition, ultrasound-induced acoustic streaming modulates the shear stress experienced by ECs, further opening the BBB.²¹¹ Low-power FUS (4.6 W) has been employed to instantly open the BBB in Alzheimer's patients and reversibly close it after 24 h without causing adverse events.¹⁸⁵ Low-intensity pulsed ultrasound combined with intravenous MBs has also been tested successfully in clinical trials for drug delivery in recurrent glioblastoma.²¹² While FUS holds promise as an emerging delivery technology, further clinical trials

The methods for crossing the BBB can be broadly categorized into active targeting and physical disruption. Active targeting involves biological carriers that use biotropy to reach and cross the BBB, as well as micro/nanomatters that use outfield manipulation and surface modification to actively target the BBB. On the other hand, physical disruption involves the reversible disruption of BBB integrity in the target region through external field stimuli. The characteristics of the three methods are summarized in Table 1. Biological carriers, such as exosomes, offer natural selectivity and BBB-crossing abilities, reducing the potential for drug toxicity and enhancing drug stability. However, challenges in production and purification need resolution.¹³⁰ Clinical trials for gene-based drug delivery using biological carriers are already underway,²²² with brain delivery as a promising direction. Artificial micro/nanomatters, such as micro/nanorobots, possess integrated versatility for drug delivery, image guidance, and therapy monitoring. Nevertheless, their biocompatibility and long-term effects require continued

Table 1. Comparison of different modes of BBB nanoparticle crossing

Method	Advantages	Limitations	Potential applications
Active targeting: biological carriers	high selectivity good crossing ability low toxicity and side effects high drug stability	complicated synthesis difficult to purify difficult to transport	gene delivery brain tumor treatment neural repair
Active targeting: micro/nanomatters	versatility spatiotemporal controllability high degree of modifiability	unknown side and long-term effects	gene delivery brain embolization therapy
Physical destruction	high efficiency of drug delivery region selectivity low drug dosage non-invasive	possible side effects unknown long-term effects complexity of equipment	neurodegenerative disease therapy brain tumor treatment

attention. Future applications may involve real-time monitoring and navigation of micro/nanomatters using advanced super-resolution medical imaging technology. Physical methods, such as FUS, enhance drug delivery efficiency and reduce drug doses, allowing the use of existing drugs without lengthy approval processes. However, potential side effects and long-term consequences make physical methods challenging for clinical implementation, necessitating careful risk assessment. Among these methods, FUS-MB, representing physical disruption, is the closest to clinical practice.

MICROSYSTEMS FOR INVASIVE DRUG DELIVERY

In addition to the previously discussed systemic route for brain drug administration across the BBB, there are alternative effective methods represented by invasive local delivery. These approaches allow for the bypassing of the BBB. The primary modes of administration that facilitate this bypass are intracerebral delivery (e.g., i.c.v. and intraparenchymal) and peripheral delivery (e.g., intrathecal and intranasal) (Figure 7). While these invasive methods are associated with potential damage, they are highly effective in the surgical treatment of conditions like malignant brain tumors, subarachnoid hemorrhage, and PD.⁹⁴ Utilizing a range of micro/nanosystems, including NPs, implantable scaffolds, hydrogels, and optical fibers, can potentially reduce the invasiveness, lower intervention risks, and enhance treatment outcomes. This section provides examples of how micro/nanosystems are employed in invasive drug delivery, both through intracerebral and peripheral routes (Figure 8).

Intracerebral administration

Intracerebral administration can be categorized into two methods: i.c.v. administration and intraparenchymal administration, based on the site of intervention. i.c.v. injection is directed into the CSF-filled lateral ventricle, whereas intraparenchymal administration involves the direct injection of implants or drugs into the brain parenchyma. Both of these approaches bypass the BBB, reducing systemic toxicity and circumventing drug metabolism in the bloodstream. However, they suffer from the drawback of slow drug diffusion in the brain parenchyma.¹⁹ Various techniques involving sustained or pressurized release have been developed to enhance drug diffusion,^{225,224} yet additional methods are still needed to minimize trauma and improve efficacy.

i.c.v. administration. i.c.v. administration stands as a precise and highly efficient technique for the direct delivery of therapeutic agents into the ventricular system. The i.c.v. administration procedure entails surgical exposure of the brain, facilitating the injection of medicinal substances into the lateral ventricles through an outlet catheter connected to an implantable reservoir.¹⁹ This method has found application in both preclinical and clinical contexts for diverse therapeutic purposes. Researchers have employed i.c.v. administration to introduce NPs,²³¹ liposomes,²³² MBs,²³³ lentiviruses,²³⁴ and siRNAs²³⁵ into mouse ventricles. In addition, Kim et al. have developed an i.c.v.-delivered MRI contrast agent, capable of penetrating the brain parenchyma through CSF circulation, enhancing the imaging of brain tumors.²²³ Notably, i.c.v. injection of mesenchymal stem cells (MSCs) has garnered recent attention in the context of AD treatment. A clinical trial using the Ommaya reservoir for continuous injection of MSCs has demonstrated both safety and feasibility.²³⁶ Ongoing research endeavors seek to optimize the therapeutic efficacy, such as by incorporating iron oxide NPs to enhance the brain retention of MSCs.²³⁷ i.c.v. administration ensures consistent, elevated drug concentrations at the target site, thereby minimizing potential side effects associated with systemic drug delivery. Nevertheless, it is imperative

to consider strategies for mitigating the inherent risks associated with this invasive procedure.

Intraparenchymal injection. Intraparenchymal administration, akin to i.c.v., involves the targeted delivery of drugs or implants directly into the brain parenchyma. However, it is important to note that drugs introduced through this method exhibit limited diffusion rates and penetration within the brain parenchyma.¹⁹ To enhance the efficiency of intraparenchymal administration, micro/nanosystems have been explored, employing sustainably released polymers for drug delivery. Natural or synthetic polymers can be crosslinked to form hydrogels for this purpose. Notably, recent research has investigated the use of injectable hydrogels for postoperative treatment of brain tumors, where the eradication of tumor cells deeply embedded in the brain parenchyma is a primary concern. Kang et al. developed hydrogels incorporating drug-laden micelles and ferrimagnetic iron oxide nanocubes, facilitating drug penetration through magnetic field-induced thermal response.²²⁴ Injectable hydrogels also find application in postoperative immunotherapy of brain tumors, as demonstrated by Chen et al., who injected chimeric antigen receptor (CAR) gene-laden nanoparticle-hydrogel into the tumor cavity to stimulate macrophages or microglia to produce glioma stem cell-specific CAR.²³⁸ In a similar vein, Zhang et al. proposed an injectable hydrogel containing tumor immune regulators for postoperative enhancement of tumor immunity in glioblastoma, thus reducing the recurrence.²³⁹ Furthermore, these hydrogels can serve as carriers for CAR-T cells, ensuring their prolonged retention and activation within the solid tumor niche.²⁴⁰ Injectable hydrogels represent a promising alternative strategy for the postoperative treatment of brain tumors.

Convection-enhanced delivery. An alternative strategy for improving the distribution of therapeutic agents within the brain post-intraparenchymal administration involves the application of positive pressure to directly inject substances into the tissue, thus enhancing their diffusion across the target region. This technique, known as convection-enhanced delivery (CED), entails the introduction of therapeutic agents through a surgically implanted catheter, utilizing an external infusion system to generate positive pressure. This pressure aids in the movement of the therapeutic agent from the infusion site into the brain ECs. CED has demonstrated its ability to augment the concentration of NPs and AAV within the brain.^{241–243} For example, Wang et al. employed CED to inject platinum anti-cancer drug-loaded NPs into glioblastoma xenograft mice, successfully circumventing temozolomide resistance.²²⁵ CED is also a valuable option for brain tumor immunotherapy. Parker et al. employed CED to deliver D2C7 immunotoxin and α CD40, resulting in a sustained anti-tumor immune response against malignant gliomas in mice.²⁴⁴ Clinical trials are underway to assess its effectiveness in patients with recurrent malignant glioma (NCT04547777). When implementing CED, it is essential to consider catheter design and material diffusion characteristics to ensure safety and efficacy.²⁴⁵

Implantable matter. Biocompatible brain-implantable matter enables controlled and sustained drug release. Various implantable forms such as extended-release wafers,²⁴⁶ hydrogels,²⁴⁷ conductive polymers,²⁴⁸ and polymer patches²⁴⁹ have been developed. Microfabrication techniques have enabled the creation of fine-structured implants. Di Mascolo et al. designed a biodegradable bilayer micromesh for independent co-loading and combination therapy with two drugs.²²⁶ Wang et al. developed microneedle patches for on-demand delivery of separate drugs, reducing glioblastoma volume and enhancing mouse survival.²⁴⁹ Microfabrication techniques also facilitate the creation of neural electrode arrays with microfluidic channels for drug delivery, electrical stimulation,

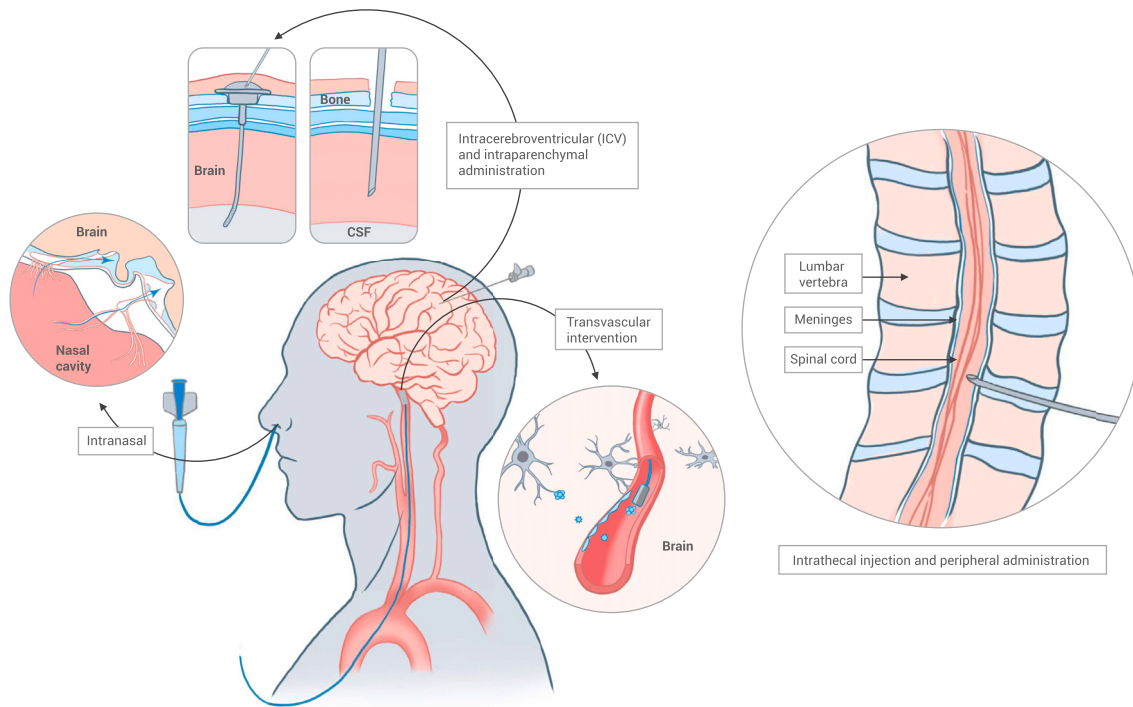


Figure 7. Scheme of microsystems for invasive drug delivery Microsystems can facilitate the invasive delivery of drugs to the brain, including intracerebral administration, intranasal delivery, intrathecal injection, and transvascular intervention. Note: this figure is modified from Furtado et al.¹⁹

and optical recording.²⁵⁰ However, this emerging field faces challenges related to device biocompatibility, neuroinflammation, long-term stability, and precise control of drug release kinetics. In addition, medical imaging, like photoacoustic imaging (PAI), is crucial for guiding implantation to avoid major blood vessels in the cerebral cortex.

Fiberbot. Optical fibers, renowned for their capacity to transmit optical signals and data, have recently found diverse applications in the biomedical domain.²⁵¹ Utilizing the thermal drawing process (TDP), nerve electrodes and microfluidic channels can be seamlessly integrated into polymer fibers alongside waveguides, enabling multiple modalities of stimulation including light, electricity, and chemistry.^{227,252,253} Canales et al. successfully created a multifunctional fiber via TDP for *in vivo* optogenetic stimulation, neural recording, and drug delivery, establishing a stable brain-machine interface in mice.²²⁷ This platform facilitated one-step optogenetics, encompassing neural recording, optical stimulation, and viral vector delivery simultaneously.²⁵⁴ Winding multiple multifunctional fibers into a spiral scaffold results in spatially expandable fiber probes, enabling 3D manipulation of brain activity.²⁵⁵ Moreover, multifunctional fiber optics exhibit promise in tumor treatment, as they can detect tumor impedance while delivering immunotherapeutic agents.²⁵⁶ Advancements in micro and nano 3D printing technology have enabled the creation of minute structures on the tip surface of optical fibers. Barbot et al. used TPP to print microfluidic channels on the fiber tip, allowing for nanoscale droplet transport.²⁵⁷ By incorporating ferromagnetic modifications to the fiber tip, this technology holds potential as a novel tool for minimally invasive endovascular interventions.²⁵⁸ This concept, termed "fiberbot," represents a controlled multifunctional fiber for navigating hard-to-reach sites for multimodal treatment.

Peripheral delivery

An alternative approach to administer substances to the brain, which circumvents BBB, is the peripheral route. This route primarily encompasses intranasal delivery, intrathecal injection, and retrograde delivery. The olfactory region in the nasal cavity provides a pathway for drugs to enter the CNS. Micro/nanomatters are introduced into the CSF via intrathecal injection, enabling an augmentation of the drug dose. When injected into muscle, micro/nanomatters can travel retrogradely along the peripheral nervous system to reach the CNS. These peripheral routes offer an alternative to the risks and limitations associated with invasive intracranial procedures.

Intranasal delivery. Intranasal delivery offers a non-invasive and patient-friendly method to bypass the BBB and reach the CNS. This approach involves the high nasal spray application of therapeutic agents, which are subsequently absorbed by the olfactory and respiratory mucosa. Drugs within the nasal cavity can access the CNS via two pathways: extracellular diffusion through the nasal epithelium and transneuronal transport along olfactory and trigeminal axons.⁹⁴ Intranasal administration boasts advantages including rapid absorption and onset of action, avoidance of hepatic first-pass effects, and excellent patient compliance. These benefits can be further harnessed by precise targeting and controlled release of micro/nanomatters. Surface-modified NPs, for instance, have demonstrated enhanced targeting efficiency in intranasal delivery.^{259,260} For example, Zhou et al. successfully delivered neurotrophic factors carried by EVs via intranasal administration to treat ischemic stroke in mice.²⁶¹ In addition, Jeon et al. devised magnetic stem cell microrobots that can be controllably guided to target tissues following intranasal administration.²²⁸ Nonetheless, the clinical application of this route still faces challenges, such as limitations in the quantity of drugs that can be administered and potential adverse reactions, including nasal irritation.

Intrathecal injection. Intrathecal injection is a minimally invasive neurosurgical procedure known for its ability to bypass the BBB and deliver therapeutic agents to the CNS. Typically, a therapeutic agent is injected into the spinal subarachnoid space through lumbar puncture, and it is then transported to the CNS via the CSF.¹⁹ Intrathecal injections have been investigated in clinical settings, primarily for conditions like leptomeningeal metastases^{262,263} and ALS.^{264,265} Research is ongoing to enhance the distribution of therapeutic agents delivered through intrathecal injections. For example, Li et al. employed positron emission tomography (PET) imaging to examine the spatiotemporal distribution of protein therapeutics following intrathecal injection, offering valuable insights into drug development for conditions such as cerebral ischemia/reperfusion injury.²²⁹ Moreover, Castle et al. discovered that the physical localization can influence gene transduction when using intrathecal injections of AAV9 to target the cerebral cortex.²⁶⁶ The use of micro/nano drug carriers holds significant promise for intrathecal drug delivery, although more research is needed to improve controlled manipulation and on-demand release.²⁷ In addition, a deeper understanding of the physiology and anatomy of the subarachnoid space is required to further enhance intrathecal drug delivery.²⁶⁷

Retrograde delivery. Retrograde delivery is a method that leverages the inherent transport mechanisms within neurons to convey therapeutic agents,

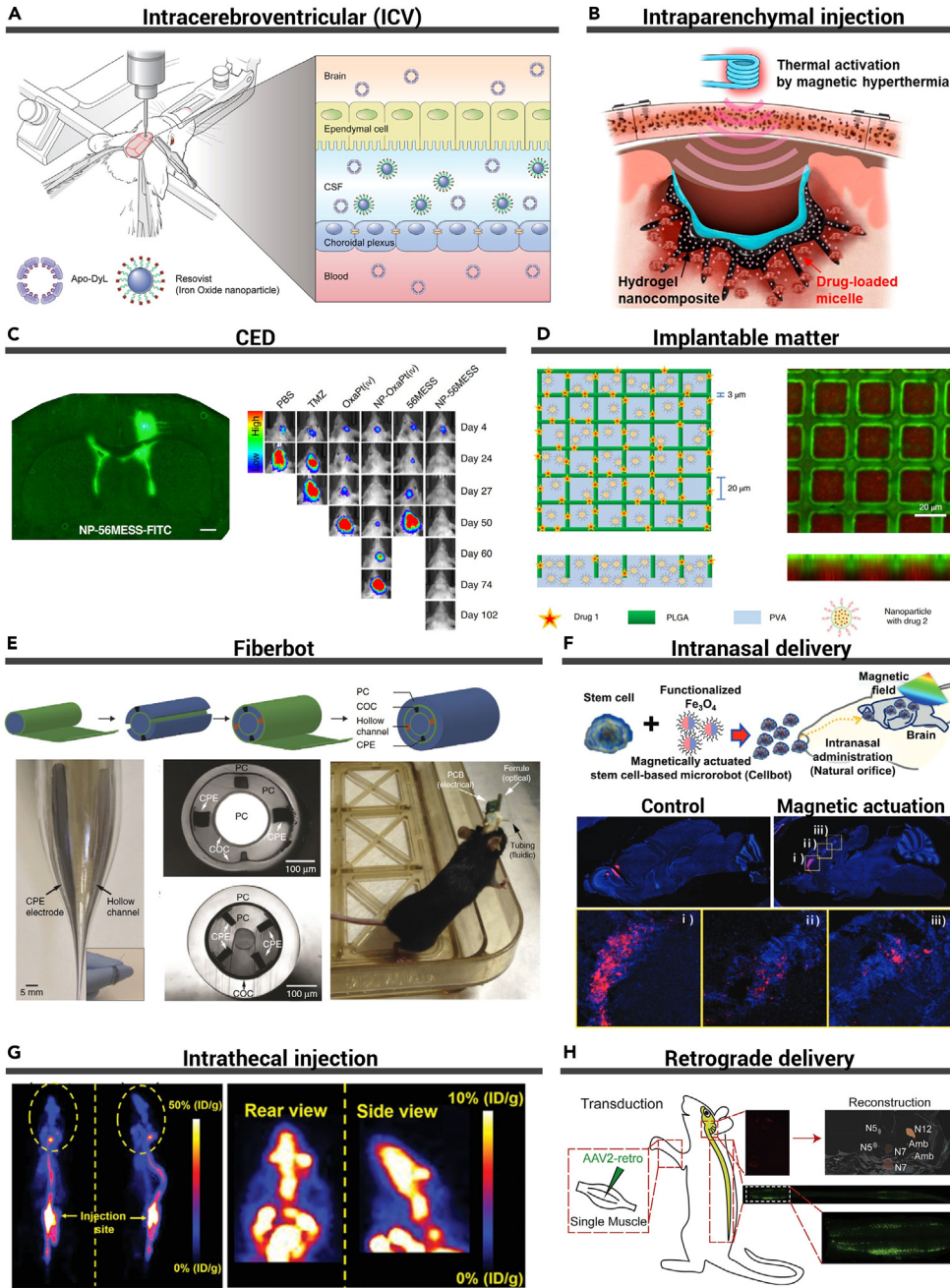


Figure 8. Microsystems for invasive drug delivery (A) Schematic illustration of stereotaxic surgery for intracerebroventricular (i.c.v.) injection, CSF-brain barrier, and CSF-blood barrier. Reproduced from Kim et al.²²³ Copyright 2020, Elsevier. (B) Schematic illustration of penetrative and sustained drug delivery to deep brain tumors by the intracortical hydrogel nanocomposite by the magnetic activation for mild hyperthermia. Reproduced from Kang et al.²²⁴ Copyright 2023, American Chemical Society. (C) Distribution of nanoparticles in the brain after CED (left). Bioluminescent IVIS images of representative mice (right). Reproduced from Wang et al.²²⁵ Copyright 2021, Springer Nature. (D) Schematic and confocal image of the structure of a micromesh, PLGA strands (green), and the PVA layer carrying RbH nanoparticles (red). Reproduced from Di Mascolo et al.²²⁶ Copyright 2021, Springer Nature. (E) Multimodality fiber probe fabrication and characterization. Reproduced from Canales et al.²²⁷ Copyright 2015, Springer Nature. (F) Schematic of the intranasal administration and magnetic actuation of cellbots (top). Sequential migration and engraftment of the cellbots from the injection site (olfactory bulb) to the cerebral cortex (bottom). Reproduced from Jeon et al.²²⁸ Copyright 2021, Wiley-VCH. (G) Representative PET images of agrin in mice at 30 min after intrathecal injection. Reproduced from Li et al.²²⁹ Copyright 2020, Wiley-VCH. (H) Reverse transport process of rAAV2-Retro to the CNS after intramuscular injection. Reproduced from Chen et al.²³⁰ Copyright 2020, Elsevier.

procedures, which present challenges in precise localization, and are susceptible to potential brain tissue damage and surgical risks. Intranasal drug delivery, in contrast, offers the advantages of ease of use and rapid drug onset, but it exhibits reduced drug delivery and absorption efficiency due to limitations related to nasal volume. The selection of the invasive approach should be grounded in a comprehensive evaluation of the patient's specific disease condition, treatment objectives, and surgical risks.

IMAGING AND TRACKING OF MICRO/NANOSYSTEMS

In the context of their application in clinical medicine, it is imperative that micro/nanomatters not only be capable of manipulation but also exhibit *in vivo* traceability. Achieving compatibility with established clinical imaging modalities is essential for ensuring the safe and controllable

tracers, or genetic material from peripheral nerves (e.g., muscles) to specific neural circuits or regions within the CNS.²⁶⁸ This approach has been instrumental in neural connectivity mapping, wherein a neural tracer, such as a viral vector, introduced at the injection site is taken up by the axon terminal. It is then transported to the soma and propagated retrogradely to the next neuron.²⁶⁹ Retrograde delivery has also demonstrated utility in targeted gene therapy for neurological disorders in both mice and primates.^{270,271} Chen et al. intramuscularly injected recombinant AAV, which can undergo reverse transport to reach the lower motor neurons of the brainstem, for gene therapy of motor neuron disease.²³⁰ However, this minimally invasive gene delivery method remains in the early stages of research, and further studies focusing on peripheral neurobiology are essential for its development and optimization.

The aforementioned invasive local drug delivery modalities represent viable strategies for surgical brain interventions, each bearing distinct advantages and drawbacks. i.c.v. drug administration facilitates even distribution across multiple brain regions, resulting in elevated drug concentrations within the CSF. Intraparenchymal administration allows for the direct delivery of a highly concentrated drug into specific brain regions, making it well-suited for treating localized lesions. Nonetheless, both of these methodologies entail surgical brain

delivery of drugs to the brain. Traditional medical imaging techniques have undergone extensive validation in pre-clinical models.²⁷² One such imaging modality for micro/nanomatters is MRI, which enables *in vivo* visualization of these entities.²⁷³ However, contemporary MRI integration with drug delivery to the brain faces challenges, such as slow imaging rates and real-time tracking limitations. In some instances, the introduction of ferromagnetic materials into micro/nanomatters is necessary to enhance MRI contrast.²⁷⁴ This has led to the exploration of combining micro/nanomatters with another magnetic-based imaging method, magnetic particle imaging, for precise localization of MNP swarms.²⁷⁵ Furthermore, radiological imaging techniques, including X-ray imaging and PET, offer powerful tools for tracking micro/nanomatters.^{276,277} Nevertheless, the limitation of ionizing radiation-induced damage to the human body is a notable concern. Ultrasound imaging shows promise as a non-invasive and cost-effective method for visualizing micro-objects.^{278,279} Wang et al. showed *in vivo* ultrasound Doppler guided magnetic swarms.¹⁹⁴ However, its utility for imaging micro/nanomatters may be constrained by position errors and sub-millimeter resolution limitations.

Given that single-mode imaging methods alone cannot fulfill the requirements for *in vivo* tracking of micro/nanomatters, there is substantial potential in

Table 2. Represented clinical trials

Drug name	Material	Conditions	Delivery route	Trial register	Status	Phase	Last update posted
EGFR(V)-EDV-Dox	bacterially derived minicell	glioblastoma, astrocytoma	intravenous	NCT02766699	recruiting	phase 1	2019
AVXS-101	AAV9	spinal muscular atrophy	intravenous	NCT04851873	completed	phase 3	2023
Skysona	lentiviral	cerebral adrenoleukodystrophy	intravenous	NCT03852498	active, not recruiting	phase 3	2022
DNX-2401	adenovirus	recurrent high-grade glioma	intraarterial	NCT03896568	recruiting	phase 1	2023
MSCs-Exos	exosome	Alzheimer's disease	intranasal	NCT04388982	unknown	phase 1, phase 2	2021
LipoCurc	liposome	glioblastoma	intravenous	NCT05768919	recruiting	phase 1, phase 2	2023
AGuIX	polymeric NPs	brain metastases	intravenous	NCT03818386	recruiting	phase 2	2022
NU-0129	AuNP	gliosarcoma	intravenous	NCT03020017	completed	early phase 1	2022
ExAblate, Definity	microbubbles	Alzheimer's disease	intravenous	NCT02986932	completed	not applicable	2018
Implantable ultrasound device SonoCloud-9 + Abraxane	microbubbles	glioblastoma	intravenous	NCT04528680	recruiting	phase 1, phase 2	2022
MTX110	NPs	glioma	convection-enhanced delivery	NCT04264143	recruiting	phase 1	2022
Nanoliposomal irinotecan	lipid-based NPs	high-grade glioma	convection-enhanced delivery	NCT02022644	active, not recruiting	phase 1	2022
APH-1105	NPs	Alzheimer's disease	intranasal	NCT03806478	not yet recruiting	phase 2	2021
Cytarabine	liposome	central nervous system metastases	intrathecal	NCT00992602	completed	phase 2	2017
GLIADEL	wafer	metastatic brain cancer	intraparenchymal	NCT00525590	completed	phase 2	2020
CD19-CAR T cells	lymphocyte	central nervous system lymphoma	intracerebroventricular	NCT05625594	recruiting	phase 1	2022

harnessing hybrid medical imaging modalities. Notably, MRgFUS has been employed in clinical settings to accomplish real-time tracking of MB. Another category of hybrid imaging that integrates ultrasound is PAI, which has recently found applications in tracking micro/nanomatters. PAI capitalizes on the contrast advantages of optical absorption and the resolution benefits of ultrasound, achieving resolutions ranging from a few microns to hundreds of microns for multiscale imaging. Various PAI systems, including photoacoustic computed tomography (PACT), optical-resolution photoacoustic microscopy (OR-PAM), and fast-scanning OR-PAM, are utilized for imaging micro/nanomatters.²⁸⁰ Wu et al. notably employed PACT for real-time tracking of microrobots within the intestines.²⁸¹ In another instance, Wrede et al. achieved real-time 3D tracking of magnetic microrobots within mouse blood vessels using optoacoustic tomography.¹⁶⁴ To further enhance resolution, Li et al. employed OR-PAM, achieving micron-level tracking accuracy.²⁸² In the pursuit of *in vivo* real-time tracking of micro/nanomatters, a synergistic approach employing various imaging methods should be tailored to the specific clinical scenarios at hand.

In invasive brain drug delivery procedures, surgeons are tasked with optimizing drug delivery efficiency while minimizing the risk of postoperative neurological impairment. In this context, advanced preoperative and intraoperative imaging assumes a pivotal role by assisting the surgeon in target area localization, procedural guidance, and ensuring precise drug delivery. Neuronavigation methods are now widely employed in various surgical scenarios, including functional neurosurgery and intracranial tumor interventions, and have become indispensable tools in the realm of minimally invasive neurosurgery.²⁸³ These neuronavigation modalities include cortical and subcortical stimulation mapping,²⁸⁴ intraoperative MRI,²⁸⁵ functional neuronavigation,²⁸⁶ navigable intraoperative ultrasonography,²⁸⁷ and fluorescence-guided neuronavigation.²⁸⁸ A burgeoning trend is the adoption of multimodal neuronavigation, which integrates and aligns multiple intraoperative image datasets to mitigate navigation and localization errors arising from brain deformation, thereby significantly enhancing intraoperative navigation

precision.²⁸⁹ The ongoing advancement of high-precision neuronavigation technology, coupled with the innovative fiberbot's neural microprobe drug delivery system, holds promise for introducing novel solutions to the realm of interventional drug delivery within the brain.

CONCLUSION AND FUTURE PERSPECTIVES

The delivery of micro/nanomatters to the brain faces significant challenges due to the tightly regulated BBB. While various approaches have been explored to cross or bypass the BBB, only a limited number of successful preclinical investigations have progressed to human clinical trials, resulting in the approval of only a handful of drugs for market release. Table 2 provides an overview of representative clinical trials in this context. Among the biological carriers, gene vectors, particularly AAV, have demonstrated remarkable progress in clinical trials for the treatment of CNS diseases. In fact, the first marketed drugs within this category are already available.^{290,291} Numerous ongoing AAV-related clinical trials encompass a spectrum of conditions, including neurodegenerative diseases, neurosensory disorders, and lysosomal storage disorders.²⁹² However, the accumulation of long-term follow-up data and the continuous refinement of carrier structures to mitigate potential risks remain imperative. Conversely, the utilization of exosomes and cellular therapies for CNS diseases is still in its nascent stages within the clinical realm, but it holds substantial promise for the future. Among artificial micro/nanomatters, NPs exhibit the most promise for clinical applications in CNS diseases, primarily due to their demonstrated efficacy in solid tumors.²⁹³ However, most ongoing clinical trials use non-targeted delivery, posing a challenge for precise BBB and lesion targeting. MBs, when combined with approved MRgFUS, have produced favorable results in clinical trials for various brain disorders.^{185,202,204} Clinical trials employing invasive methods for micro/nanomatters brain delivery are underway, but the scarcity of clinical trials relative to successful preclinical experiments highlights translation challenges. Molecular drugs offer established solutions for brain diseases, with

comprehensive reviews available for further reference (see Nance and co-workers^{94,107}). This review emphasizes the emerging role of micro/nanosystems.

Brain-targeted micro/nanomatters encounter several clinical translation challenges, including the following.

- (1) Optimizing size, surface properties, and functionalization is crucial for improving BBB permeability. Furthermore, achieving precise localization within the brain and preventing off-target effects are imperative. Exploring the integration of multiple surface modifications for sequential targeting (BBB, brain parenchyma, neurons) holds great promise.
- (2) Minimizing potential toxic effects, inflammatory responses, and tissue damage caused by these micro/nanomatters or their degradation products is of paramount importance. A comprehensive understanding of the micro/nanomatters' pharmacokinetics, residence time, and clearance routes is essential to optimize drug delivery and reduce systemic distribution. Moreover, thorough evaluation of long-term biocompatibility and the possibility of accumulation in the brain or other organs is necessary.
- (3) Precise control over the movement and distribution of these micro/nanomatters in the brain is essential. This necessitates the development of methods for actively actuating and manipulating micro/nanomatters *in vivo*. The choice of the actuation method should align with the specific application scenario. Utilizing multiphysics field propulsion and manipulation holds promise in this regard.
- (4) Real-time imaging guidance is vital for achieving spatiotemporally controlled delivery of micro/nanomatters in the brain. The integration of multimodal imaging techniques with advanced neuroimaging methods, including ultra-high field MRI, fast scanning PAI, and magnetic particle imaging, holds the potential for real-time localization of these micro/nanomatters.
- (5) Micro/nanomatters encounter issues related to inefficient transport over extended distances within the brain. The considerable distance between the point of intervention and the target regions in the brain can lead to substantial losses if these particles are transported directly in the bloodstream. In response, the concept of the fiberbot has emerged, aiming to deliver and release the payload locally into the brain's blood vessels through transvascular intervention.
- (6) The ultimate goal is to develop micro/NPs with multifunctional capabilities, encompassing image navigation, motion control, targeted delivery, and outstanding biocompatibility for clinical use. These controllable micro/NPs share traits with robots and have the potential to evolve into micro and nanorobots.²⁹⁴ However, this advancement is accompanied by significant challenges.

Preclinical models, such as animal models, may not faithfully replicate human brain physiology or disease conditions. Consequently, observations of efficacy and safety in preclinical studies might not always translate to human patients. Bridging this gap and substantiating the safety and efficacy of brain-targeted drugs in human trials represents a crucial phase in the clinical translation process. The advent of *in vitro* BBB microsystem models signifies a breakthrough in brain research. These models, with the ability to mimic the intricate structure and function of the BBB, offer numerous advantages, including enhanced precision in drug screening, a deeper comprehension of disease mechanisms, and potential applications in personalized medicine. Several future prospects for μ BBB models include.

- (1) Advancements in microfluidic device fabrication techniques, including the integration of 3D printing and organ-on-a-chip technologies, hold the potential to create more realistic and sophisticated BBB models.
- (2) The combination of the BBB microfluidic model with other organ models, such as the liver or gut, can facilitate the study of systemic drug effects and enhance the prediction of drug pharmacokinetics^{295,296}
- (3) Scaling up the BBB microfluidic model for high-throughput screening and ensuring long-term stability and reproducibility are crucial for its broader utilization.
- (4) Manipulating cell distribution within the BBB microfluidic model provides a robust method for investigating the spatial organization and

cell-cell interactions of the BBB. Techniques like cellular patterning, control of cell ratios, and their combination with advanced imaging can offer valuable insights into barrier function, neuroinflammatory processes, and disease mechanisms. Existing methods for cell patterning using ultrasound fields may be adapted for use in the μ BBB model to achieve vascular patterning⁷⁴

Brain interventions, like neural stimulation or drug delivery, necessitate tools that are small and precise to minimize harm to delicate neural tissue. The emergence of fiber-based robotic devices, known as fiberbots, offers a promising avenue for attaining this level of precision in brain interventions. The application of fiberbots in brain interventions provides several advantages over conventional methods.

- (1) Fiberbots enable precise drug delivery to specific brain regions through minimally invasive surgery, mitigating the risk of complications and side effects.
- (2) They facilitate the stimulation or recording of individual neurons or small neural circuits, enhancing our understanding of brain function.
- (3) Fiberbots support other minimally invasive procedures like biopsies or tissue sampling without necessitating large incisions or bulky instruments.

Nevertheless, the development of fiberbots presents challenges, including the need for advanced control systems and real-time monitoring of their position and movement. In addition, using biocompatible materials for fiber construction is vital to prevent damage to neural tissue.

Brain drug delivery, an advanced approach for treating CNS disorders, confronts regulatory and ethical challenges during clinical translation. These factors are pivotal in ensuring patient safety and treatment success. Rigorous regulation should be applied throughout the entire process of brain drug development, encompassing safety assessment, drug manufacturing quality, and validation of therapeutic efficacy. Concurrently, government and regulatory authorities should streamline the drug and medical device approval system to foster innovation in brain drug delivery. Given the elevated risk associated with brain drug delivery, clinical trials should give thorough consideration to legal and ethical concerns, including informed patient consent, privacy, long-term safety, and societal impact. Further research is warranted to enhance the design and optimize the utilization of these technologies for safe and efficacious brain interventions.

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AUTHOR CONTRIBUTIONS

Conceptualization, Z.M. and G.-Z.Y.; writing – original draft, M.T.; writing – review & editing, Z.M. and G.-Z.Y.; supervision, G.-Z.Y.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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