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Brain-on-a-chip: an emerging platform for studying the nanotechnology-biology interface for neurodegenerative disorders

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Abstract

Neurological disorders have for a long time been a global challenge dismissed by drug companies, especially due to the low efficiency of most therapeutic compounds to cross the brain capillary wall, that forms the blood-brain barrier (BBB) and reach the brain. This has boosted an incessant search for novel carriers and methodologies to drive these compounds throughout the BBB. However, it remains a challenge to artificially mimic the physiology and function of the human BBB, allowing a reliable, reproducible and throughput screening of these rapidly growing technologies and nanoformulations (NFs). To surpass these challenges, brain-on-a-chip (BoC) – advanced microphysiological platforms that emulate key features of the brain composition and functionality, with the potential to emulate pathophysiological signatures of neurological disorders, are emerging as a microfluidic tool to screen new brain-targeting drugs, investigate neuropathogenesis and reach personalized medicine. In this review, the advance of BoC as a bioengineered screening tool of new brain-targeting drugs and NFs, enabling to decipher the intricate nanotechnology-biology interface is discussed. Firstly, the main challenges to model the brain are outlined, then, examples of BoC platforms to recapitulate the neurodegenerative diseases and screen NFs are summarized, emphasizing the current most promising nanotechnological-based drug delivery strategies and lastly, the integration of high-throughput screening biosensing systems as possible cutting-edge technologies for an end-use perspective is discussed as future perspective.

Keywords Brain-on-a-chip, Blood-brain barrier, Nanomedicine, Neurodegenerative diseases

Introduction

Neurodegenerative disorders (NDs) include a broad category of brain diseases caused by progressive death of neuron cells with the decline of brain function, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease, amyotrophic lateral sclerosis (ALS), among other incapacitant neurological conditions [1, 2]. Worldwide it is estimated that NDs represent 6.3% of disease burden, with an annual economic weight surpassing more 700 billion dollars just in the U.S [3]. Despite all the progress and effort that has been made in the last few decades regarding the research in NDs, the complexity of the human brain that veils the underline mechanism of neurological diseases, combined with the low efficiency of a minority of drugs able to cross the blood-brain barrier (BBB) have halted the advance of significant breakthroughs in neurosciences and medicine [4]. Thus, and despite the widely effort to study NDs, there are no currently available treatments to stop or reverse

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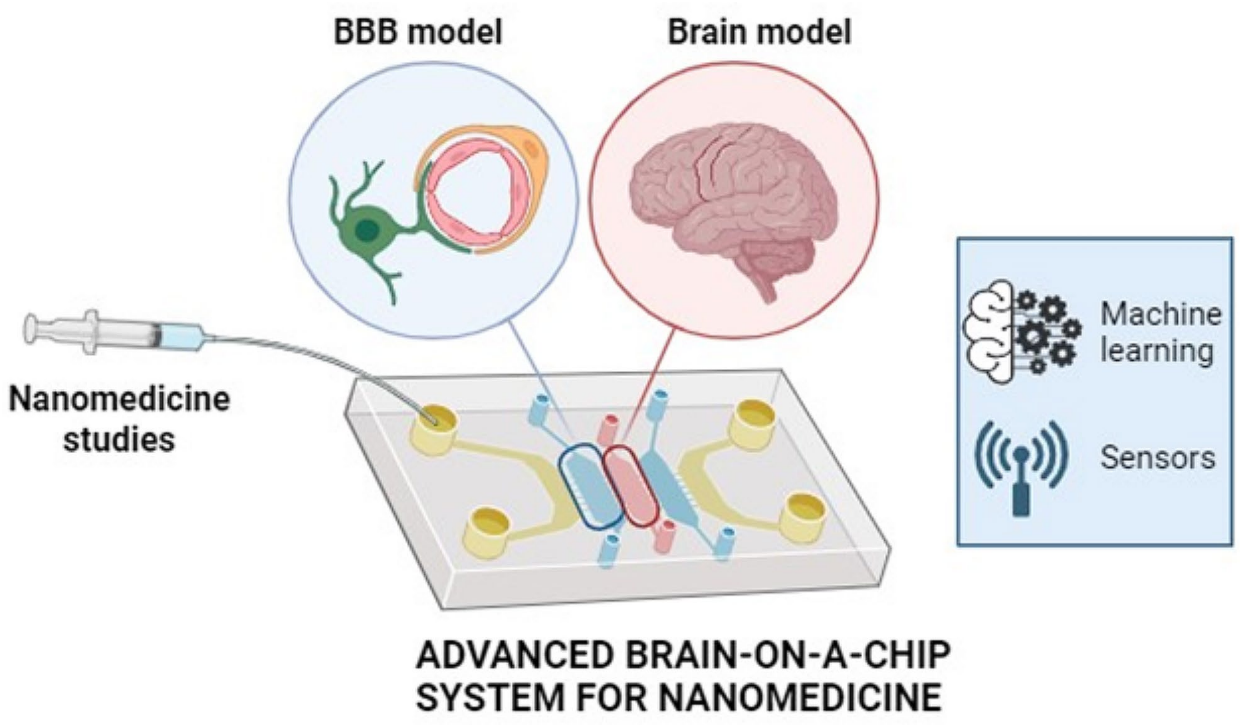
Graphical abstract



the degeneration of a diseased brain [5]. At present, the incomplete understanding of NDs is based on the complex role of a variety of factors that include oxidative stress, protein aggregation and misfolding in the peripheral nervous system (PNC) and central nervous system (CNS), generally attributed to aging and/or genetics [6, 7].

Typically, two-dimensional (2D) culture models and animal models are used as standard techniques for drug development and mechanism research [8, 9]. However, none of these gold standard methodologies can perfectly mimic the physiological settings and complexity of the human brain, creating a technological gap that needs to be addressed. Specifically, 2D systems are in vitro models that enable fast and less costly modelling experiments. However, there are several limitations to emulating the complex structure of the human brain, including cell-cell interaction and organized three-dimensional (3D) neuronal populations [2], proven to be ineffective in the prediction of neuro-cytotoxicity and drug screening [10]. On the other hand, animal models, such as ND-induced mouse, can provide more valuable physiological and mechanistic insights for the understanding of molecular and cellular pathogenesis. Nonetheless, due to species-specific differences between humans and animals, the prediction of drug/nanoformulation (NFs) efficacy and, accurately recapitulate of ND, are limited [9]. For instance, for the investigation of PD and degeneration of dopaminergic neurons, mouse models can be chemically induced with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or genetically modified (α-synuclein transgenic mouse) [9, 11]. In the case of AD, includes transgenic, knock-in, and injection of amyloid-beta (Aβ) β and tau proteins, which are established as the two main pathogenic factors [12]. Although their interest, none of the genetically engineered models can perfectly reflect the human-like pathologies, and the chemically induced models only can replicate some aspects of the neurodegenerative human diseases. For instances, it has been reported that for AD-related pathologies using genetically modified models, from over two hundred studies, only two have produced medicines that were approved for clinical use [1], representing less than 1% of successful rate. Additionally, these models rise ethical issues and should be avoided at all costs to minimize the animal suffering or discomfort, by applying the 3Rs principle (Replacement, Reduction, and Refinement) [13]. Another in vitro strategy is the 3D brain model, a cell culture system that through co-culture of neuronal and endothelial lineage cells can recapitulate certain aspects of the in vivo spatial and functional complexity of the human brain. In this tissue-like models, the mimetic system tends to show a more similar behavior, including gene expression, chemokine production and drug response, not observed in

2D systems [14]. Typically, these 3D models are modeled in an extracellular matrix (ECM) hydrogel of natural, synthetic, or combined materials that provide structure and plays a key-role in several aspects of cell self-assembling, including adhesion, elongation and growth, resulting in tissue regeneration and organ development [15]. Representative natural 3D neural systems have been developed with natural materials include gelatin, hyaluronic acid, alginate, collagen type I, fibrin and Matrigel [16, 17]. Among the synthetic materials, biopolymers such as polyethylene glycol (PEG), polyacrylamide (PAM), and poly(2-hydroxyethylmethacrylate) (p-HEMA) are used for 3D brain models [1, 18]. Lately, 3D brain organoids using decellularized extracellular matrix (dECM) scaffolds have also been described in literature [18]. This last example of biomaterial refers to human or animal brain tissue, such as porcine, with the removal of immunogenic cellular components via decellularized technologies that have shown interesting results in proliferation, migration and neural differentiation [19]. Generally denominated by 3D brain organoids, these in vitro 3D models can derive from human pluripotent stem cells (hPSCs), including embryonic or induced PSCs, which can provide a suitable bioplatform to study pathological features of NDs [2]. Although the considerable advantages over 2D cultures, 3D models also have some limitations, namely lack of physical compartmentalization between neuronal and vascular tissues and, dynamic mechanical stresses derived from fluidic stresses, such as shear stress, that is necessary, for instance, for the auto-regulation of the endothelial barrier and angiogenesis, so important in the representation of BBB [20].

Recently, organ-on-a-chip (OoC) has emerged as an advanced microfluidic platform combined with 3D tissue culture techniques, with the goal of recapitulating human physiology and homeostasis at a lower cost and higher reproducibility [21]. This new methodology bypasses the ethical concern regarding the use of animals for testing of human products, which is in line with the 3 Rs' animal principle, and it can also overcome the low capability of animal tests to predict the effects of drugs and NFs. For these reasons, OoCs have been receiving great attention from the pharmaceutical industry, which is seeking new ways to improve the drug development process and personalized treatment, or to obtain better model diseases to understand their mechanisms and etiology [22]. In this regard, nanomedicine, which enables the design of NFs that can be engineered as active targeting drug nanocarriers, i.e., peptides, aptamers, miRNA or antibodies, allowing for targeting, loading and controlled delivery of a high range of medicines to specific organ/tissues of the body, has received great attention in recent times [4]. This, in general, results in the inhibition of common side-effects of free drugs and maximizes the therapeutic efficiency. Moreover, the same NF can be designed to simultaneously target, diagnose and treat diseased cells, such as neurodegenerative disorders, fulfilling the theranostic performance (diagnose+treatment). Furthermore, some of these engineered NFs, with sizes lower than 100 nm, have shown the ability to cross the BBB, since they can be endocytosed more easily by cells [4]. However, as in the case of the development of new drugs, their clinical translation has been scarce, due to issues related to the lack of robust preclinical tools to screen, in the initial development stages, their clinical safety, toxicity or neuroprotection [8].

Based on these new technological and scientific advances, herein, we summarize the ultimate studies based on brain platforms – brain-on-a-chip (BoC), including neuronal and BBB models, that mimic the mechanistic, physiology and pathology of NDs. Firstly, the main challenges to model the brain are outlined, then, relevant brain models and current BoC platforms are described. Particularly, BBB-BoC will be discussed in more detail, due to its utmost importance in the screening of brain-targeting NFs for NDs. Examples of BoC platforms are summarized, including their ability to recapitulate the neurodegenerative diseases and screen NFs (Fig. 1). Lastly, the integration of biosensing technology and artificial intelligence is debated as a possible cutting-edge technology to expand the state-of-the-art, fostering BoCs as standard in vitro screening platforms.

Advanced microphysiological systems for modeling CNS

In vitro challenges to model the brain

Before discussing the in vitro modeling of the human brain (CNS), it is important to pinpoint the main challenges that such a multiscale complex system represent. Overall, the structural complexity of the brain includes neurons, glia cells (such as astrocytes, microglia and oligodendrocytes) and pericytes, forming the neurovascular unit (NVU), immune cells and brain vascular endothelial lineage cells [23, 24], (Fig. 2A). It is estimated that the human brain contains 100 billion neurons that form the neuronal network with glial cells, transmitting information via synapses, in a process known as neurotransmission [9]. This creates a dynamic connection, stronger or weaker depending on the frequency of the synapses, between neurons that change overtime in a process known as neural plasticity. Besides that, it is estimated that neurons alone count more than 500 subtypes with specific cell-cell interactions. Moreover, the brain contains more than 250 different regions holding their unique functionality, microenvironment, cellular composition and architecture, which is very challenge to recapitulate in vitro [3]. Furthermore, the brain is connected to multiple brain subunit systems, namely brain

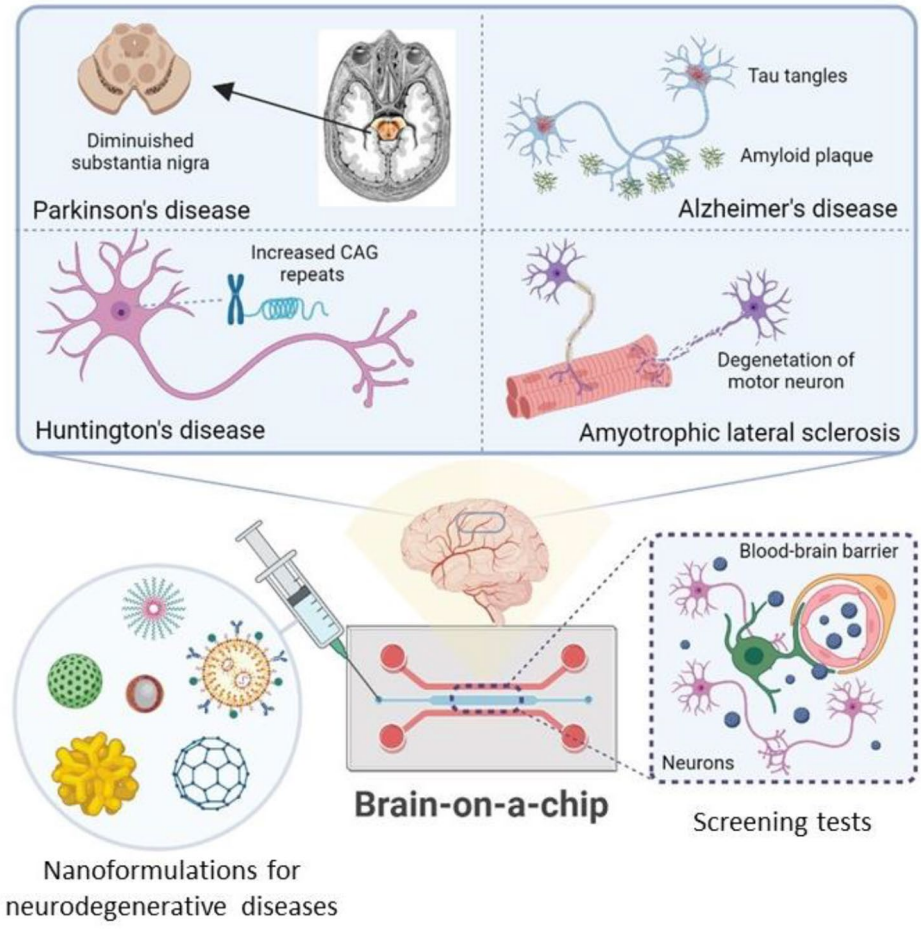


Fig. 1 Representation of brain-on-a-chip platforms to emulate pathophysiological features of neurodegenerative diseases, serving as preclinical screening tool of novel theranostic nanoformulations

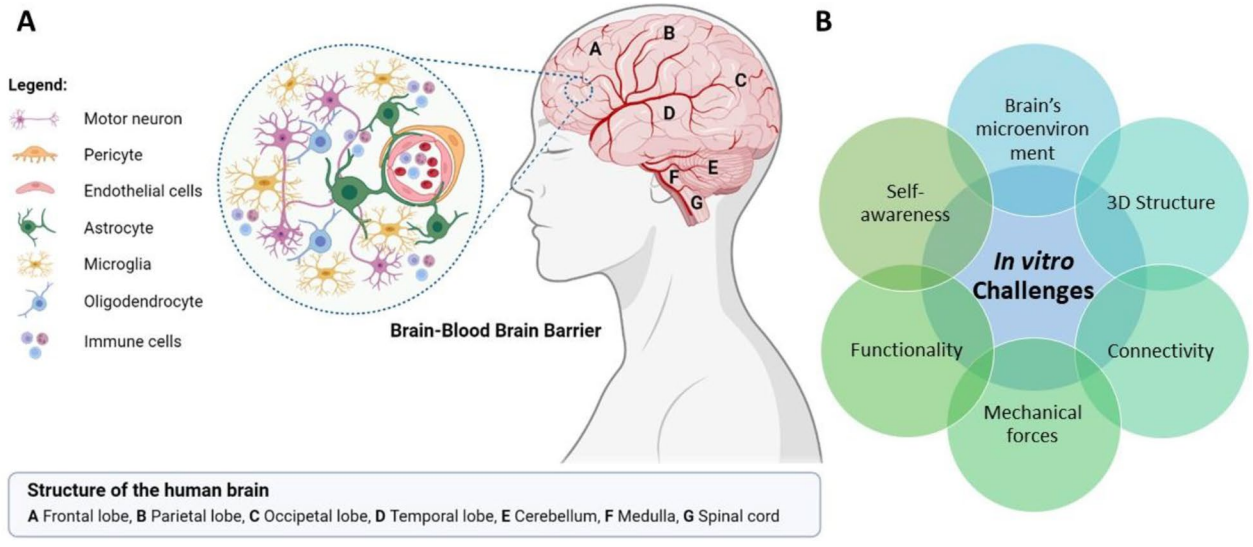


Fig. 2 (**A**) Illustration of the structure of the brain, particularly the brain-blood brain barrier microenvironment and cell-cell interactions; (**B**) The main challenges for the accurate reproduction of the brain as an in vitro model

endocrine system, choroid plexus, glymphatic system, vasculature system and barriers (i.e., BBB, blood-CSF barrier, blood-spinal cord barrier and arachnoid barrier), which are crucial for the functionality and homeostasis [3, 25]. In a recent perspective paper published by Maoz, B.M., 2021 [3], the sole-author reflects on the main challenges that should be overcome to generate an in vitro model representative of the human brain. Among the abovementioned complexity of the brain microenvironment, structure, connectivity, mechanical forces and functionality (Fig. 2B), the author also highlights a particularity of the human brain that is still mostly not understood – how neuronal electrical activity between brain cells can be translated in higher-order functions, such as self-awareness or consciousness. All these challenges show the necessity to develop in vitro bioplatforms able to close replicate parts or totally the human brain, surpassing one of the most technological and engineering challenge that we current face – the puzzling of the human brain. Overall, its achievement can shed light not just on our understanding of the brain and neuronal diseases, but ultimately on us, as humans and our evolution as specie.

Organ-on-a-chip to surpass the CNS modeling challenges

The concept of an OoC – a biomimetic microsystem that comprises organ models with microfluidic devices, was first introduced in 2010 by Huh et al., in the group led by Donald E. Ingber [26], where the authors published a novel microfluidic device compressing a human alveolar-capillary interface to recapitulate the physiological and functionality of a breading lung. Since then, a variety of OoCs have been developed to mimic different human physiological conditions and single organs, such bone, brain, eye, heart, liver, lung, skin, vascular systems, among others (topic reviews can be found elsewhere [10, 22, 27, 28]); and progressing to the development of multi-organ models and human-on-a-chip (HoC) [28]. Beside the ability that OoCs show to recapitulate vascular perfusion in a dynamic microenvironment, these biomimetic microsystems have additional advantages over traditional animal-based models and cell-based models, namely the advantage to recreate tissue-tissue interfaces, organ-relevant complexity and functionality, allowing the on-demand application of physical, mechanical and biochemical stimuli found in the human body [3, 10, 28, 29]. Thus, comparing with animal models, OoC have the added benefits to enhance the prediction results, increase the test duration, improve reproducibility, as well as to reduce fabrication cost and operation complexity [10]. For these reasons, OoC provide a modeling bioplatform for CNS and screening of novel drugs and NFs with direct benefits that are not achieved by conventional in vitro platforms, such as the shear forces found in the brain and brain barriers, allowing to test different concentrations and timepoints [3], and evaluate the drug/NF ability to cross the BBB and target brain diseased cells in representative models of CNS.

Table 1 gathers the main advantages that BoCs have to surpass the CNS modeling challenges in comparison with the in vitro gold standard methodologies.

To achieve a significant neuromimetic platform that exceeds the capabilities of traditional in vitro methodologies, the BoC device must address some of the unique CNS modeling challenges described in Table 1 [3]. One of the most significant advantages over conventional cell cultures, is the inclusion of microfluidic-based platforms that provides the opportunity to recreate the shear stress on the BBB, enhancing the BBB properties over static models. Due to the crucial role that BBB presents as a brain's gatekeeper, static models present limitations to emulate BBB, such as their ability to predict drug effects. Although BoCs show several advantages in mimicking of brain models and interconnectivity to brain sub-units

Table 1 Advantages of the brain-on-a-chip devices to surpass the challenges of the in vitro modeling of CNS

Main challenges to modeling CNS	Brain-on-a-chip advantages over gold standard models	References
Brain's microenvironment	Possibility to incorporate native ECM materials, with similar mechanical properties as the brain, e.g., stiffness, permeability, shear stress, etc. Allow spatiotemporal control of cellular and biochemical environment. Co-culture of motor neurons, interneurons and glia brain-tissue ECM, with incorporation of immune cells. On-demand design of the brain's microenvironment to replicate disease and/or healthy brain models.	[1, 2, 30–32]
3D structure	Incorporation of co-culture brain cells in 3D models that mimic the structure of the brain in its unique 3D architecture, mimicking different brain regions, such as forebrain organoids, cerebral organoids, or midbrain organoids.	[33, 34]
Connectivity	Enables the creation of neuronal architecture with controlled patterns and connections. Allows to control neuronal direction, anatomic connection, and dynamic network. Integration of multiple brain regions (e.g., cortex, thalamus, cortical, hippocampus, striatal, among others). Integrations of brain sub-units (e.g., BBB, retina, mucosa, among others). Possibility to study organ-organ interactions (e.g., brain-gut-on-a-chip, multi-organ-on-a-chip, etc.).	[20, 35–39]
Functionality	Possibility to monitor cell-cell interactions as basic building block of the brain, measure electrophysical activity, homeostasis, processing sensory inputs and control outputs. Allows automation and monitoring in continuous and at real time by the integration of (bio)sensors and readouts.	[3, 32, 40]

compared to static models, the complex brain multifunctionality is still not fully represented with today's technology. As stated by Maoz, 2021 [3], in in vitro systems, the emulation of brain capability to monitor homeostasis, process sensory inputs and outputs, present self-awareness, consciousness and cognition goes beyond any currently "platform-on-a-chip". Some of these aspects are being studied using advanced neuronal platforms for computer software [41, 42] and controlling flight simulations [43]. But most of the BoC devices used a simplified definition of brain's functionality targeting neurons and measuring their electrophysiological activity [44]. By so, in Sect. Brain-on-a-chip (including BBB), the state-of-the-art of BoC platforms will be discussed in more detail, including some technological limitations, as the incorporation of physiological brain aspects and sub-units, including immune system.

The unique physiology of the brain and the role of BBB in the CNS

Due to its vital importance and cell activity, the brain has evolved with an extra protection system of blood vessels (i.e., BBB), which prevents toxins and other harmful substances from reaching it. This protective BBB, a highly selective membrane with low permeability, is also the main reason for the difficulty in creating effective drugs that are able to cross this barrier and target brain cells [9]. The efficiency of the BBB is so high, that it is estimated that 100% of large-molecule drugs do not cross the BBB, and just 2% of small-molecule drugs with mass below 500 Da are able to cross it [45]. It is generally accepted that only substances with a low molecular mass and lipophilic behavior can bypass the BBB freely (Fig. 3A) [45, 46]. However, most drugs have a higher molecular mass, which in general demands an endogenous transport system for the molecules to move across the BBB. Examples are transport-mediated transcytosis, receptor-mediated transcytosis, cell-mediated transcytosis, and absorptive transcytosis [47, 48], (Fig. 3A). Briefly, transport-mediated transcytosis or protein-mediated transport, is based on proteins that are responsible for carrying specific molecules. Among those proteins are glucose transporter type 1 (GLUT-1) and large neutral amino acid transporters (LAT), playing a crucial role in the delivery of several molecules to the brain [49]. Receptor-mediated transcytosis uses the activation of brain endothelial cell to transport endogenic molecules and is considered a promising approach to delivery drugs into the CNS [49]. Examples of this transcytosis are transferrin receptor (TfR) [50], low-density lipoprotein receptor (LDLR) [51], insulin and insulin like growth factor receptor [52], albumin receptor [53], lactoferrin receptor [54] and low-density lipoprotein-receptor related protein 1 and 2 (LRP1 and LRP2) [55]. Another important via to cross the BBB is the adsorptive-mediated transcytosis that is based on the electrostatic interaction between the negatively charged membranes of the brain endothelial cells and the positively charged molecules (usually polycationic proteins) [56]. Overall, transcytosis is being used as key-strategy to get nanomaterials through the BBB into the CNS and to enhance the efficiency of drug delivery.

Anatomically, the BBB is composed of endothelial cells, pericytes embedded in basal lamina and astrocytes

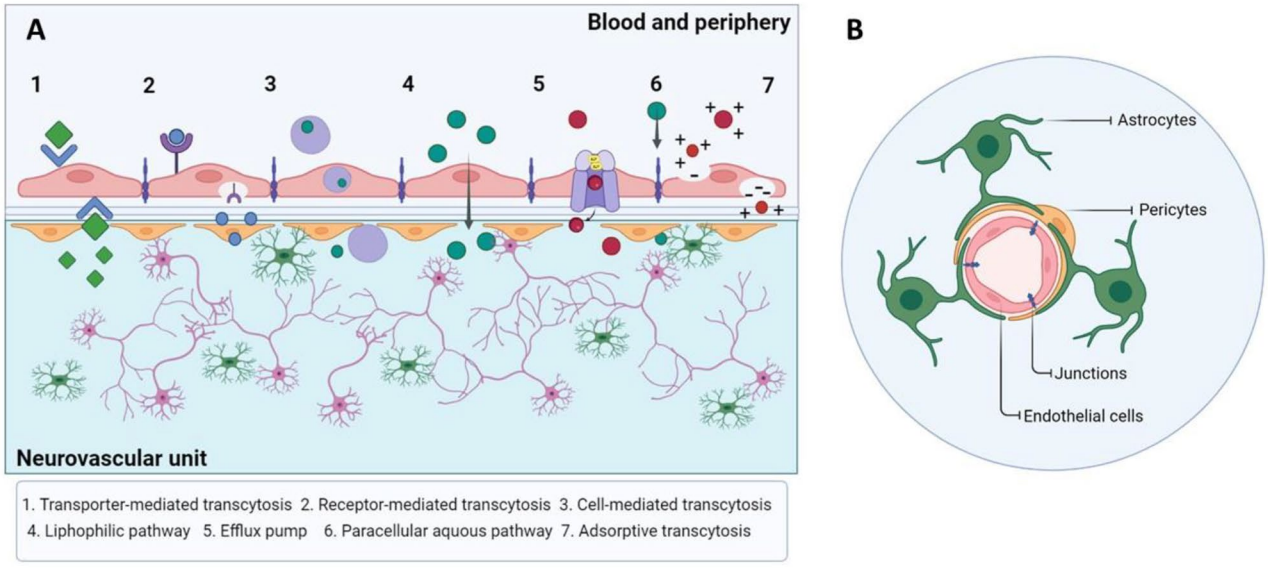


Fig. 3 (**A**) Representation of the distinct mechanism that molecules can use to cross BBB. (**B**) Illustration of the cells that compose the BBB (3D representation)

end-feet, touching the abluminal side of the brain vessels (Fig. 3B). Endothelial cells are the core structure of the cerebral blood vessels that interact with other CNS's cells. Their morphology and function differ from peripheral endothelial cells, by presenting tight and adherent junctions, no fenestration but small transcellular pores, which restrict free diffusion and rapid exchange of molecules between the blood and brain. Also, they present specific transporters that regulates the flow of specific substrates, creating a protective barrier to molecules that reaches the brain [47]. Pericytes are vascular mural cells embedded in basal lamina, wrapping around endothelial cells and creating a close communication and regulation between them. Their main function is to help to regulate the BBB permeability, cerebral blood flow, clearance of toxic metabolites, neuroinflammation and stem cell activity [57]. Astrocytes, also known as star cells, are the most numerous glial cells (which also includes microglia) and have several functions in CNS, including dynamic signaling for clear waste, regulation of the vascular function, hemostasis, balance of neuroimmune response, brain blood flow and support to the BBB [47]. Although there is still discussion about the exact role of astrocytes in BBB, it is consensual that the BBB is formed through the coordination between endothelial cells, pericytes and astrocytes. Microglia are also a type of glia cell that acts has primary innate immune cell, i.e., specialized population of macrophages, and is found in the brain after colonize it in the early stage of the brain's development [58]. Their main function is the immune surveillance of the CNS, synaptic pruning and phagocytosis of cellular debris, dead neurons and pathogens [59]. For years, studies on the BBB were focused on the contribution of endothelial cells, especially when using 2D cell culture and animal models [60]. Undeniably, these early studies have contributed to the understanding of cell lineage [61], expression of endothelial markers [62], thigh junctions [63, 64], efflux transporters [65], receptor systems [66], among others scientific discoveries. Just recently, the importance of the other cell types located in the brain tissues was acknowledged and added to the models [67, 68], where the BoC and its development have a great impact. Thus, to preserve the interaction between vascular endothelial and neuronal cells, fully replicating the NVU establishes a new benchmark for developing innovative in vitro BBB-BoC models.

Brain-on-a-chip (including BBB)

As abovementioned, BoC devices take advantage of the OoC technological approach, which has the potential to create an accurate and simple-to-use preclinical model tool, by decoupling a complex organ, such as the brain, into different cellular structures while maintaining their interconnections. This approach allows for the precise assessment of drug molecules and/or drug nanocarriers along the different tissues, unveiling new interactions between them that are essential for the development of new therapeutic strategies for neurological diseases. Also, the possibility of integrating biosensors into it could extend its monitoring and workability for longer periods (more details in Sect. Integration of biosensing systems in BoCs). Most of the recent developments in BoC follow one of two main categories, depending on their high-throughput abilities: (i) BoC that mimics the 3D brain tissue environment (i.e., material, cell types and physiological stimulation) [32, 37], or (ii) BoC that simulates cell-to-cell or organ-to-organ interactions with interconnected multichip systems [38]. Some of these studies have also been dedicated to create BoC devices that are useful for mimicking the BBB structure, addressing the issue of transport across the endothelial layer with a porous membrane and allowing communication with brain cells [8].

An example of this advanced BBB-BoC was achieved by Brown and co-workers, 2018 [69], where a human BBB microfluidic model (named as μHuB) was developed using human cerebral microvascular endothelial cells (hCMEC/D3) and human astrocytes, using a commercial microfluidic platform, Fig. 4A. Wherein, the authors verified relevant shear stresses with expression of phenotypical tight junction markers, such as Claudin-5 and Zonula occludens protein 1 (ZO-1), with size-selective permeability close to BBB models (10 and 70 KDa). In another study, Pediaditakis et al., 2022 [32], reported the development of a BBB-brain human organotypic microphysiological system containing endothelial, pericytes, glia and cortical neurons to recreate critical aspects of neuroinflammation, serving as brain-chip model able to study novel therapeutics for brain diseases, and to understand cell-cell interactions and BBB function during neuroinflammation. In this study, the researchers report similar transcriptomic profiling to human adult cortex by using next-generation sequencing data and databases of signature genes, reporting identical proinflammatory cytokines, and compromised BBB permeability when exposed to tumor necrosis factor alpha (TNF-a), Fig. 4B.

Indeed, the lack of efficient drugs that can cross the BBB and treat NDs is a main concern in neurosciences and medicine. In this regard, nanomedicine, which enables the design of NFs that can be engineered as active targeting drug nanocarriers, allowing for targeting, loading and controlled delivery of a high range of active substances to specific organ/tissues of the body, has received great attention in recent times, especially to enhance the targeting and efficiency of drug delivery into the CNS [4]. However, their clinical translation has been scarce, specially due to the lack of robust in vitro CNS models able to screen at the development phase some strategies

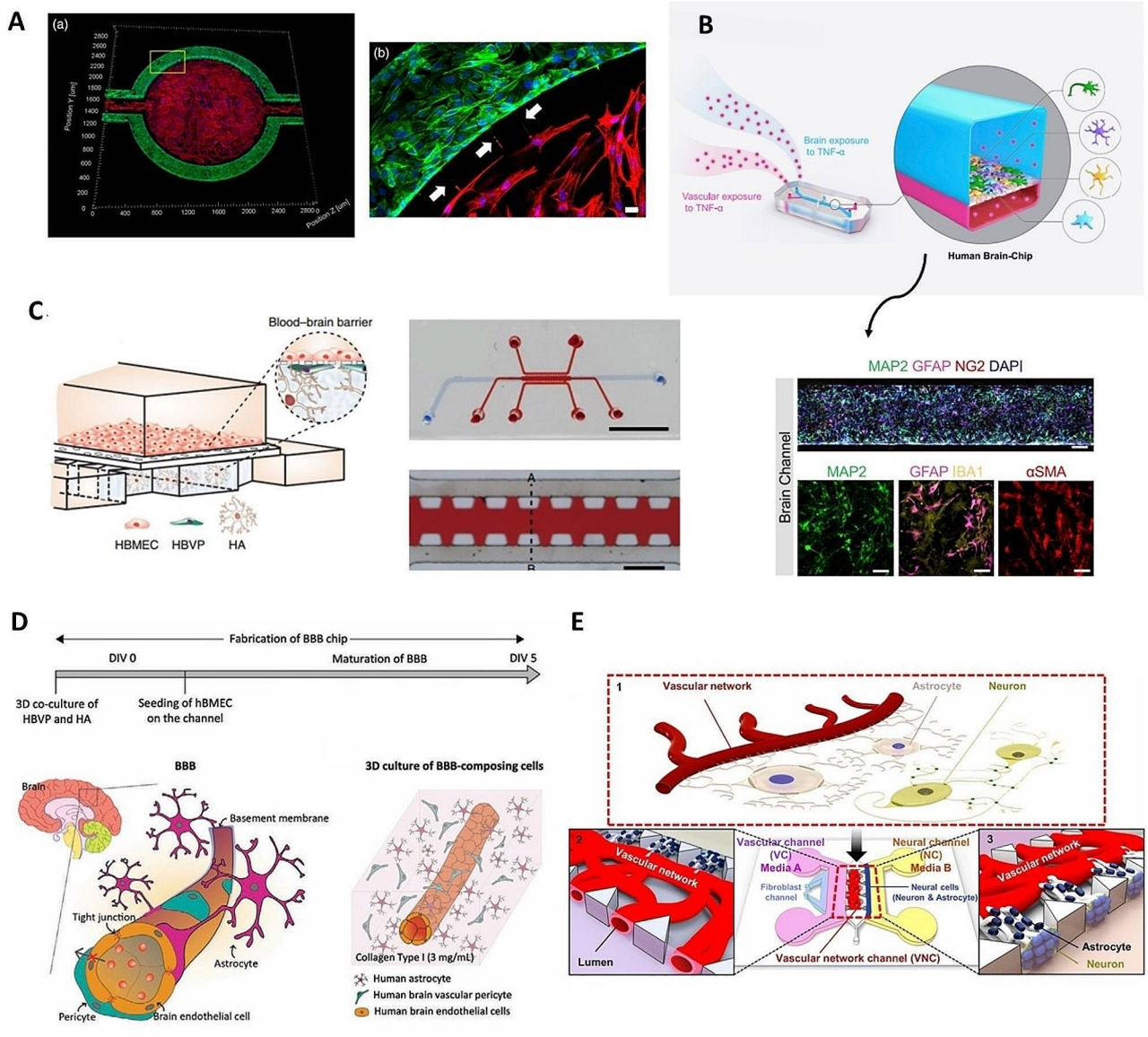


Fig. 4 Examples of different designs of microphysiological systems developed to recapitulate the human brain. **A.** (**a**) Co-culture of hCMEC/D3 and human astrocytes using a commercial microfluidic device (μHuB) containing an apical compartment seeded with hCMEC/D3 (green) and a central compartment containing human astrocytes (red), (**b**) Zoomed-in of the pore membrane (3 × 3 × 50 μm, w × h × d) that connect apical and central compartments. Scale bar = 20 μm. Reproduced with permission [69]. Copyright 2018, Wiley. **B.** Illustration of the Brain-Chip device designed as a two-channel microengineered chip incorporating brain endothelial-like cells (BBB) cultured on the bottom channel and separated by a porous membrane from neurons (MAP2, green), primary human brain astrocytes (GFAP, magenta; IBA1, yellow), pericytes (aSMA, red), and microglia (top channel representing the brain model). Scale bar = 50 μm. Reproduced with permission [32]. Copyright 2022, iScience. (**C**) Schematic illustration of the microengineered human BBB model containing human brain microvascular endothelial cells (HBMECs) (top red channel) seeded on top channel separated by a porous membrane from the human astrocytes (HAs) and human brain vascular pericytes (HBVPs) (bottom blue channel) (scale bar = 100 μm). Reproduced with permission [70]. Copyright 2020, Nature. Open access. (**D**) In vitro 3D BBB triculture model assembled in hydrogel. Illustration of the timeline for the fabrication of the human BBB-device, comprising endothelial cells, pericytes and astrocytes. Reproduced with permission [71]. Copyright 2022, Wiley. Open access. (**E**) Self-assembled in vitro 3D neuro vascular unit (NVU) platform. Endothelial cells (ECs) are enclosed within fibrin gel, facilitating the development of vascular networks within the matrix. Neurons and astrocytes are then seeded in the neighboring channel alongside the vascular networks. Reproduced with permission [37]. Copyright 2017, Nature. Open access

related to their BBB-crossing, clinical safety, toxicity and neuroprotection [8]. In a study published by Ahn et al., 2020 [70], a microphysiological platform was designed to recapitulate key-structures and functions of the human BBB to 3D mapping the distributions of NFs in the vascular and perivascular regions (Fig. 4C). The authors reported a mimicking BBB-brain model that besides similar structure and function, key gene expressions, low permeability, and 3D astrocytic network with reduced reactive gliosis and polarized aquaporin-4 distribution, revealed a precise capture of NPs distributions with distinct cellular uptakes and BBB penetrations through receptor-mediated transcytosis. The authors pinpoint the advantage of the developed bioplatform to serve as NFs screening tool in comparison with animal models, particularly: (i) the BBB-brain model allowed time-lapse sampling and end-point fluorescence-activated cell sorting (FACS) analysis to quantify 3D nanoparticle (NP) distribution at the BBB; (ii) the compartmentalized structure of the BBB chip allowed the separated measuring of molecules in each space and BBB penetration; (iii) evaluation of the targeting efficacy of NPs at cellular levels; as well as (iv) depth mechanistic understanding of the interactions between the BBB and NPs at cellular levels [70]. This study is an example of the capableness that microphysiological platforms to screen novel NFs in an early stage of development and optimization prior to clinical trials, fostering the engineering of brain-targeted delivery systems for neurological disorders.

In the given examples, BBB is typically represented as a 2D endothelial vascular monolayer separated from the brain model through a porous membrane or pillars, and the structural design of the device assembled as vertical or "sandwich-like" (Fig. 4B-C) or planar parallel (Fig. 4A) design. In these approaches, endothelial cells may be placed onto the porous membrane (with or without glia cells) or grown in a distinct compartment to establish monolayers featuring a vascular system. Yet, blood vessels can also be formed in 3D architecture within hydrogels, as a 3D-tubular design (Fig. 4D), either with or without perivascular cells integrated into the hydrogel matrix. An example of this methodology is the work performed by Seo et al. [71], showing the fabrication of a human BBB model by coculturing BBB-composing cells within a 3D hydrogel matrix (Fig. 4D). The authors first validated the BBB model through the analysis of the expression of BBB-specific markers, BBB permeability with and without administration of inflammatory cytokines and BBB-opening agents. After the BBB validation, the authors extended their research using this 3D BBB-model as a BBB-glioblastoma-platform to study drug delivery and BBB-associated chemosensitivity. Another example for 3D BBB assembling strategy is the work developed by Bang et al. [37], presenting a self-assembled in vitro neurovascular unit (NVU) platform with a 3D model of the BBB (Fig. 4E). This framework can accurately replicate the in vivo BBB microenvironment, complete with ECM. Leveraging the intrinsic processes of vasculogenesis and angiogenesis, endothelial cells can autonomously organize to establish vasculature. In this work the authors report that this methodology mirrors natural vascular development in vivo, resulting in enhanced BBB functionalities with potential application in the screening of medicines that targets the brain for NDs.

Although these latest 3D-BBB in vitro models resemble with more accuracy the in vivo human brain, most of these studies lack in the representation of the peripheral immune system (i.e., immune cell migration and interaction across the BBB) in response to severe injury and diseases. Indeed, the immune system has a synergetic and preponderant role in the regulation of the BBB, and vice-versa, which affects the CNS during health and disease [72]. Some of these BBB-immune interactions include: (1) the transport of cytokines and substances with neuroinflammatory properties; (2) traverse of immune cells through the BBB by tightly regulated process of diapedesis; and (3) inflammatory conditions, trauma injury and AD, which increases immune cell entry into CNS [73]. The process of immune cells moving out of the bloodstream, known as diapedesis or extravasation, involves multiple steps, namely: (i) tethering and rolling of the immune cells along endothelial cell surface, (ii) activation by recognizing chemokines immobilized on proteoglycans on the surface of endothelial cells, (iii) firm arrest of the immune cells on the luminal surface of the endothelial cells, (iv) polarization and crawling to find endothelial junctions, and (v) diapedesis across the endothelial barrier [74]. This complex process is characterized by the sequential interactions between adhesion molecules and signaling molecules present on both the vascular endothelial cells and the immune cells [72]. Thus, the in vitro modelling of immune cell trafficking across BBB, requires reliable culture systems that faithfully replicate the unique characteristics of the BBB, including the continuous interaction with components of the NVU. Also, it has been shown that the presence of shear flow in in vitro BBB models emulates unique T-cell crawling behavior observed in in vivo imaging studies [75]. So, advanced BBB in vitro models should be combined with sophisticated microfluidics and live cell imaging [72]. The development of BoC as a highly tunable in vitro system integrated with immune systems will be greatly beneficial for the advancement in the understanding of brain diseases and development of novel drugs/NFs.

Engineered brain-targeted delivery systems

With the purpose to find new ways to early diagnose and treat brain diseases, including NDs, several advanced materials and technologies have been developed. Among them, engineered brain-targeted delivery systems are one of the most promising strategies. These nanosystems, based on nanotechnology approaches to synthesize nanometric materials (organic and/or inorganic) are cornerstone in some unique attributes that make them optimal candidates to enhance brain-targeting delivery, namely (i) nanometric size, (ii) ability to encapsulate higher amount of therapeutic molecules, (iii) stimuli-responsive drug release, (iv) ease functionalization with targeting molecules, e.g., antibodies, peptides, aptamers, nucleic acids, etc., (v) enhance of its half-life into the body, (vi) imaging enhancement, and (vii) multifunctional therapeutic applications, among others [28, 46].

Designed to cross in a controlled and non-invasive manner, most of these NFs are being engineered to brain-target and deliver therapeutic molecules using diverse materials, namely lipidic, polymeric, carbon-based, silica, among others (Table 2). As abovementioned, these NFs can be used for single or combined applications, such as controlled drug delivery and imaging enhancement, or even, combining therapeutic and imaging functionalities in single nanosystems for theranostics approaches.

Among the materials used to develop NFs, *Lipid-based nanoparticles*, which include liposomes, solid-lipid NP and nanostructure-lipid NP, are one of the most studied and promising developed materials as brain-target drug delivery systems. For instance, liposomes, composed by one or more lipidic bilayers, were the first nanosystem applied for drug delivery and widely used since their discovery in the 1960s, due to their recognized biocompatibility, biodegradability, ability to cross the BBB due to their excellent endocytosis, drug loading capability and delivery efficiency, among others [47]. The most common transport routes of liposomes are carrier-mediated (cationic or PEGylated liposomes) and receptor-mediated transcytosis [76]. Examples of these NFs include the study of Zhang et al., 2019 [77], which developed a bio-inspired liposome with surface modification using a short non-toxic peptide derived from $A\beta_{1-42}$ to targeting exchangeable apolipoproteins to transverse BBB via transcytosis in mice. In another study, Kuo and Wang, 2014 [78], developed liposomes loaded with neuron growth factor (NGF) and lactoferrin to be used against Aβ-peptide and treat AD. The authors reported that the liposome formulations worked as efficient drug carriers (72–90% NGF, 48 h) able to cross the BBB, emulated as HBMECs/HAs monolayer in transwell, and inhibit the Aβ-induced neurotoxicity, promising pharmacotherapy for AD (Fig. 5A). Solid-lipid NP (SLN) is another type of lipid-based NF, considered as one of the safest and cheapest drug nanocarriers to cross the BBB and treat NDs safely and effectively [49]. Generally, it is formulated from lipid or modified lipid (triglycerides, fatty acids, or waxes) precursors and surfactant to improve the colloidal stability of the particles in aqueous solutions. An example of SLNs for drug brain delivery is the work of Neves et al., 2021 [79], which presents SLNs loaded with curcumin (an anti-inflammatory and antioxidant compound with neuroprotective activity) and functionalized with transferrin to potentially treat neurological disorders, i.e., depression, Alzheimer's, Parkinson's and Huntington's diseases. The results shown a curcumin encapsulation a round 65% in SLNs and functionalization of transferrin around 70–75%. Permeability studies using transwells with hCMEC/D3 cells monolayers revealed a 1.5-fold higher permeation of curcumin in transferrin-functionalized compared to non-functionalized nanoparticles. Indeed, recent studies have pointed out biomolecules or herbal formulations that can help to prevent or be used as adjuvants to treat NDs. Among them are curcumin, quercetin, resveratrol, piperine, Omega 3, *Ginkgo biloba* and *Nigella sativa* [80], which typically shows anti-inflammatory, antioxidant,

Table 2 Different types of nanoparticles used as brain-targeted drug delivery systems, including mean sizes (nm), representative delivery cargo and targeting approach

Nanomaterial	Size (nm)	Delivery cargo	Targeting approach	Refs
Liposome	50–500	Doxorubicin, dithranol, neuron growth factor, curcumin, and ginsenoside Rb1.	AD's peptide, lactoferrin, transferrin, anti-transferrin receptor antibody (OX26), and glucose transporter 1 (GLUT-1).	[77, 78, 93–95]
Solid lipid NP	10-1000	Rhodamine B, donepezil, curcumin, docetaxel, paclitaxel, and doxorubicin.	Apo E, transferrin, arginine-glycine-aspartic (RGD), cationized albumin, and angiopep-2.	[49, 79, 82]
Polymeric NP	10–500	Rasagiline, tramadol, dopamine, donepezil, and piperine.	Transferrin, insulin, Apo E, OX26, lactoferrin, and adenosine.	[83–85, 96]
Carbon dots	2–12	Glycine-proline-glutamate, curcumin, branched-PEI, and memantine hydrochloride	BBB passive-crossing, transferrin, and Ab peptide.	[86, 97, 98]
Mesoporous silica	20–500, with porous 2–20 nm	Doxorubicin, leptin, pioglitazone, amyloid-b antibody, clioquinol and IgG.	Apo E, glucose transporter (GLUT), RGD peptide, lactoferrin, angiopep-2, and low-density lipoproteins.	[47, 90–92]

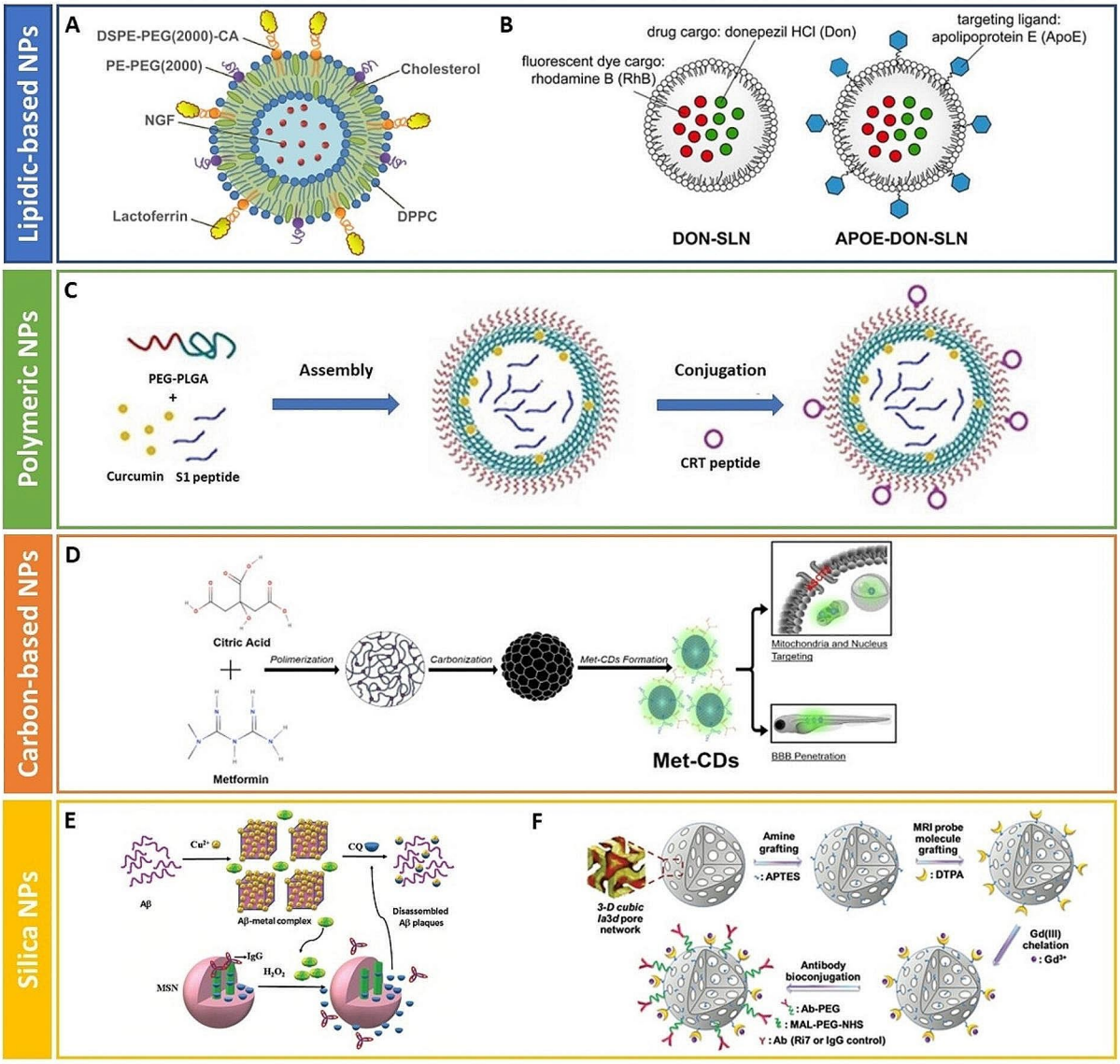


Fig. 5 Illustration of some representative engineering brain-targeted delivery systems, including lipid-based nanoparticles, (**A**) Liposomes loaded with neuron growth factor and lactoferrin to treat AD using BBB model cultured in transwells. Reproduced with permission [78]. Copyright 2014, Elsevier, and (**B**) Solid lipid NPs functionalized with Apolipoprotein E (Apo E) as receptor-mediated pathway and loaded with donepezil and rhodamine B, using BBB model cultured in transwells. Reproduced with permission [82]. Copyright 2020, MDPI. Open access., Polymeric nanoparticles, (**C**) PLGA NPs conjugated with peptide CRT and loaded with curcumin in AD mouse brains. Reproduced with permission [85]. Copyright 2017, Impact Journals LLC; Carbon-based nanoparticles, (**D**) Carbon dots derived from metformin (Met-CDs) with theranostic potential and BBB targeting using zebrafish model. Reproduced with permission [86]. Copyright 2021, Elsevier; Silica nanoparticles, (**E**) Mesoporous silica nanoparticles with H_2O_2 responsive controlled release for Alzheimer's diseases. No permeability tests across BBB are presented. Reproduced with permission [91]. Copyright 2012, Wiley, and (**F**) Antibody-conjugated mesoporous silica NP for brain-BBB cell targeting, assessed after intravenous injections in the mouse. Reproduced with permission [92]. Copyright 2017, Royal Society of Chemistry

antiproliferative and antiangiogenic activities. However, their bioavailability is often low due to their poor body absorption and hydrophobicity [81]. In this regard, lipid-based NFs represent a promising engineered drug nanocarrier to enhance their bioavailability, which is in line with the EU Pharma strategy concerning the availability, accessibility, and affordability of medicinal products. Another example of SLNs developed to cross BBB and successfully deliver donepezil (anti-Alzheimer's drug) was achieved by Topal and co-workers, 2020 [82]. In this study, the authors show the enhanced permeability of SLNs through BBB in transwells by using Apolipoprotein E (Apo E) as receptor-mediated pathway, with an increase of 1.5-fold higher delivery of drug in comparison with non-functionalized NPs (Fig. 5B).

Polymeric nanocarriers, can be developed from a series of monomers (natural and/or synthetic), using a variety of polymerization techniques and tuning properties [56]. Among synthetic NPs, Poly(Lactic-*co*-Glycolic Acid) (PLGA), Poly-e-Caprolactone (PCL), Poly(Alkyl Cyanoacrylate) (PACA), Poly(Ethylene glycol) (PEG), Poly(Lactic Acid) (PLA) and Poly[Triphenylamine-4-vinyl-(P-methoxy-benzene)] (TEB) are the most commonly used [83]. However, synthetic polymers can be sometimes restricted by their cost, lower degradation rate and, in some cases, toxicity. Alternatively, natural polymers, including polysaccharides and proteins, such as chitosan, alginate and pectin [84], emerged as promising alternatives. A common example of a synthetic polymeric NP is obtained with PLGA, constituted by monomers of glycolic and lactic acids, and approved by the Food and Drug Administration (FDA) agency for biomedical applications. In a study published by Huang et al., 2017 [85], PLGA NPs conjugated with brain targeting peptide calreticulin (CRT), to target transferrin receptor, and curcumin was developed for Alzheimer's disease (Fig. 5C). The authors reported that CRT-conjugated PLGA NPs have decreased Aβ levels, reactive oxygen species (ROS), TNF-a and Interleucina 6 (IL-6), as well as improved super oxide dismutase (SOD) and synapses in AD mouse brains.

Carbon-based nanoparticles, such as graphene, graphene oxide (GO), carbon dots (CDs) and carbon nanotubes (CNTs) have been also exploited as promising agents for biomedical applications, including drug delivery and imaging [47]. Among them CDs are one of the most promising for brain-targeting nanocarrier due to their small size that allows passive crossing through BBB, high drug capability, tunable properties, natural photoluminescence and colloidal stability. Cilingir and coworkers, 2021 [86], have developed CDs derived from metformin (Met-CDs) and citric acid using a microwave-assisted method (Fig. 5D). The resulting CDs show a mean size of 7 nm with spherical shape and luminescent properties enabling bioimaging experiences as biomarkers. Also, the Met-CDs exhibits BBB penetration property along with mitochondrial and nucleus targeting using zebrafish models, which due to their available functional groups have the potential to be conjugated with therapeutic drugs, completing the theranostic ability. Nevertheless, carbon-based nanoparticles raise biosafety concerns among researchers, since they are mostly non-biodegradable materials with apparent dependent-properties that lead to cytotoxic effects (strongly related with factors, such as size, morphology, mass basis, surface property, and concentrations) [87]. For instance, some studies demonstrated that the cytotoxicity of CNTs is greatly influenced by the mass basis, to which single walled CNTs show to have higher cytotoxicity than multi-walled CNTs [88]. To minimize this effect and increase their biocompatibility, some groups have functionalized their carbon-based nanomaterials with polymers (such PEG) or biomolecules, which also improved their long blood circulation time and lower their uptake by the reticuloendothelial system (RES) [89].

Silica nanoparticles are another type of material to synthesize NFs. They are stable, low toxic and inert nanocarriers that can be loaded with fluorescent probes to be used as imaging agents and/or surface-functionalized with drugs, by the presence of their silanol groups [90], and used as theranostic nanosystems. Among their advantages, mesoporous silica NPs (MSNs) present pore sizes between 2 and 40 nm that makes them attractive for higher drug cargo capability, due to their high pore volume and surface area [47]. An example of the early studies showing the potential of MSNs for neurological disorders was published by Geng et al., 2012 [91], where the authors reported the development of smart MSNs with H_2O_2-responsive controlled release for AD treatment (Fig. 5E). The MSNs systems were functionalized with arylboronic esters that in the presence of the cellular oxidant, H_2O_2, released loaded IgG and clioquinol (a chelate) to decrease Aβ aggregate deposits in AD. However, no studies were presented regarding their ability to cross the BBB. In another study, the potential of MSNs (developed with two different sizes, 50 nm and 160 nm) was demonstrated by Bouchoucha et al., 2017 [92], with the development of an antibody-conjugated MSNs for brain-BBB cell targeting (Fig. 5F). Wherein, the authors conjugate Ri7 antibody through a PEG linker and gadolinium chelate (Gd-DTPA) to serve as MRI imaging enhancer, assessed after intravenous injections in mouse. A highest specific uptake was found with 50 nm Ri7-MSNs, and the results show the ability of Ri7-MSNs to internalize in brain models, both in vitro (2D cell uptake) and in vivo, and potentially serving for theranostic application in NDs.

Although a multitude of NFs are being in development as engineering brain-targeted delivery systems, which has led to several FDA-approved trials, the lack of robust and appropriate in vitro platforms for a rapid translation application is still an unmet need. As exemplified in this section, most of the BBB permeability studies are presented using in vivo models (mouse, mice and zebrafish) and/or in vitro models, as transwells (i.e., static flow models). Few studies have been published using BoC models for the screen of nanomedicines, mainly due to the infancy phase and acceptance of these microphysiological models as standard preclinical screening platforms. An example of such studies is the work published by Ahn et al., 2020 [70], already described in Sect. The unique physiology of the brain and the role of BBB in the CNS, where a neuromimetic platform was designed to

recapitulate key-structures and functions of the human BBB to 3D mapping the distributions of NFs in the vascular and perivascular regions (Fig. 4C). The NFs used in this study was an engineer high-density lipoprotein (HDL)-mimetic nanoparticles with apolipoprotein A1 (eHNP-A1), previously developed by the authors [99]. In this previous study, the authors also employed an innovative translational nanomedicine strategy by combining a microengineered vasculature system for in vitro screening of eHNP-A1 comparing with in vivo vascularized tumors, using murine model. Another example of the use of BoC for the permeability evaluation of NFs designed to cross the BBB and treat brain diseases, is the recent study published by Palma-Florez and co-workers, 2023 [100]. In this study, a BBB-on-a-chip platform with an integrated trans-endothelial electrical resistance TEER measurement system was used for the evaluation of the permeability performance of targeted gold nanorods (GNR) for theranostics of AD. The GNR functionalized with polyethylene glycol (PEG), angiopep-2 peptide (Ang2) and D1 peptide, labeled as GNR-PEG-Ang2/D1, was designed to cross the BBB with proven potentiality to disaggregate Aβ in in vivo and in vitro studies. Overall, the authors reported the BoC device as a viable alternative to animal experiments to evaluate the brain permeability of NF in physiological environment with human cells, with advantage in the lower cost, reproducibility, and avoidance of ethical constrains. Thus, modeling NDs and physiological brain barriers on a chip, although not yet fully adopted as a preferential methodology over the conventional in vivo models to screen the performance of NFs, represents a field of high importance to bridge the gap between neuroscience and material engineering in a free-animal way.

Modeling neurodegenerative diseases on a chip

Alzheimer's disease (AD)

AD is a progressive ND with at least two known pathological traits, the presence of Aβ neurofibrillary within extracellular senile plaques and tangles of hyperphosphorylated tau proteins that create aggregates inside neurons [9, 101]. This leads to progressive memory loss, language deterioration and functional impairment. Although the exact AD pathological mechanism is still not understood, the amyloid hypothesis has been the main theory accepted by the research community over the last three decades [102], since the work of John Hardy and David Allsop, in 1991 [103]. In this study, the authors found a pathogenic mutation on chromosome 21 in the Aβ precursor protein (APP) gene that was correlated with AD. However, some recent AD treatments based on amyloid targeting, such as Aβ-targeting monoclonal antibodies, have failed in clinical trials, although showing promising results in reducing free plasma Aβ concentrations by more than 90%, such as the case of Solanezumab (Eli Lilly). Other examples of drug failure during phase III in clinical trials are Crenezumab (Roche/Genentech/AC Immune) and Aducanumab (Biogen Idec) [102]. This shows the urgent need to develop better in vitro AD models able to underline the complex mechanisms of AD pathogenesis and screen novel therapeutics in development [9]. In this regard, one of the first advanced microfluidic biomodels was performed by Park and colleagues, 2015 [104], which developed a microfluidic chip comprising multiple 3D neurospheroids, formed in concave microwells, to mimic the in vivo brain microenvironment (healthy and AD) and applying constant interstitial flow (Fig. 6A). Wherein, the authors reported the neurotoxic effects of Aβ, with loss of cell viability and increased neural destruction and synaptic dysfunction, associated with in vivo AD. More recently, Palma-Florez and co-workers, 2023 [100], developed a BBB-on-a-chip integrated with micro-TEER to evaluate the permeability of multi-functionalized theranostic gold nanorods for AD. The advanced microfluidic device was fabricated with a BBB model containing astrocytes, pericytes and endothelial cells, and integrated with TEER measuring system integrated close to the endothelial barrier to monitor the barrier integrity and functionality. The microdevice showed significant expression of the BBB tight junctions and neurovascular network, which was used to screen the NFs capability to cross BBB when the NFs were functionalized with Ang2 peptide (Fig. 6B).

Parkinson's disease (PD)

PD is a progressive ND that affects movement with symptoms that include uncontrollable tremors, slowness, posture imbalance and rigidity, and that leads to severe disability and death [1, 9]. The neuropathological hallmarks for PD are the formation of intracellular inclusions, i.e., Lewy bodies, and the accumulation of a-synuclein protein aggregates [101]. It has been hypothesized that accumulation of α-synuclein promotes neuroinflammation with activation of microglia with an increase of proinflammatory cytokines (e.g., IL-1β, IL-6, INF-γ and TNF-α) and degeneration of dopaminergic neurons and astrocytes [101, 105]. Additionally, there has been evidence that PD patients show microgliosis, astrocytosis and infiltration of T-leukocytes, pinpointing the need to develop appropriate in vitro systems with co-culture of dopaminergic neurons, astrocytes and microglia cells [9]. An example of these microphysiological systems was recently developed by Pediaditakis and co-workers, 2021 [106], where a human brain-BBB chip was fabricated with dopaminergic neurons, astrocytes, microglia, pericytes and microvascular endothelial cells cultured under dynamic flow (Fig. 6C). The authors report that their BoC model with a-synuclein fibrils was capable to recapitulate

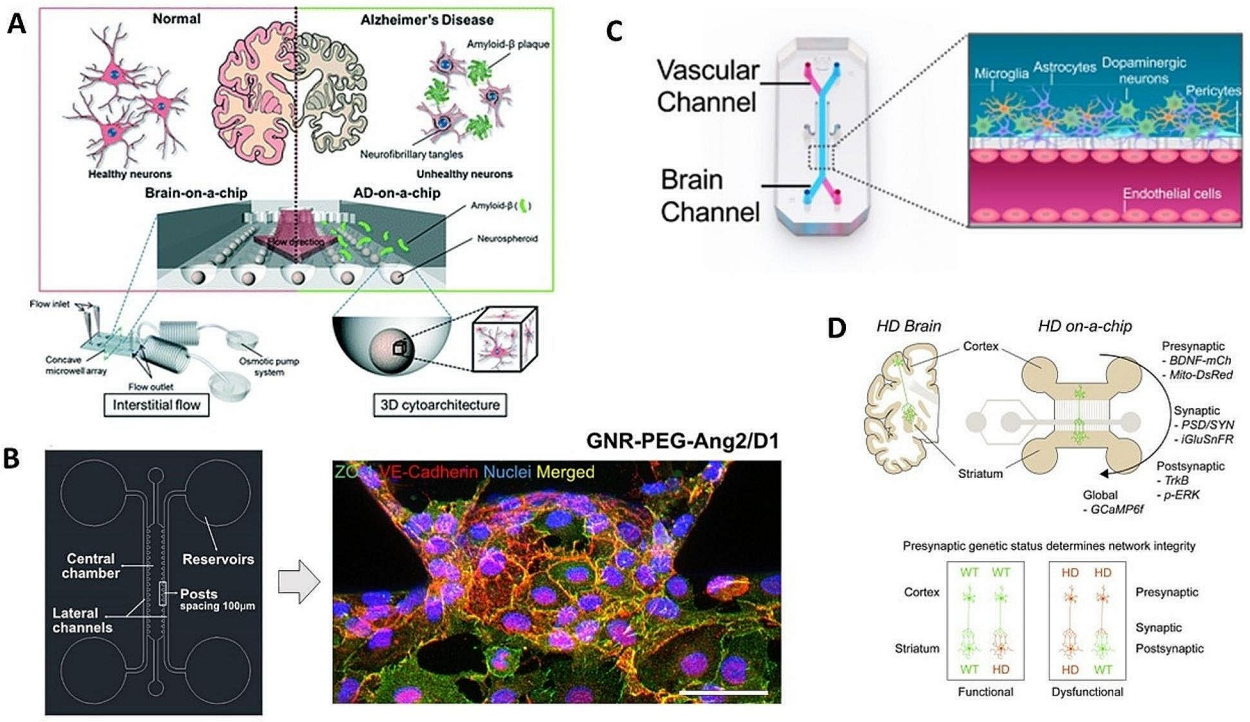


Fig. 6 Examples of representative modeling neurodegenerative diseases on a chip. (**A**) Microfluidic chip comprising multiple 3D neurospheroids to model Alzheimer's diseases. Reproduced with permission [104]. Copyright 2015, Royal Society of Chemistry. (**B**) BBB-on-a-chip integrated with micro-TEER to evaluate the permeability of theranostic gold nanorods for Alzheimer's diseases. Reproduced with permission. Scale bar = 50 μm [100]. Copyright 2023, BioMed Central. (**C**) Human Brain-BBB chip to study Parkinson's diseases. Reproduced with permission [106]. Copyright 2021, Cell Press. (**D**) Huntington's disease-on-a-chip model with reconstructive corticostriatal network. Reproduced with permission [107]. Copyright 2018, Cell Press. Open access

key aspects of PD, including mitochondrial impairment, neuroinflammation, compromised barrier function and phosphorylated a-synuclein, ideal for screening novel therapies for PD.

Huntington's disease (HD)

HD is an inherited neurodegenerative disorder caused by a mutation in the huntingtin gene, located in exon 1 of chromosome 4, with increase repetitions of the trinucleotides CAG (Cytosine-Adenine-Guanine) above diseases threshold (36 or more) that codes glutamine protein [1, 9]. The accumulation of toxic proteins in neurons and disruption of the corticostriatal circuit are considered the main neuropathology of HD [107]. HD patients show loss of striatal and cortical neurons, mainly in the motor or premotor areas, and consequently difficulty to control the body. Thus, the in vitro modeling of HD is being attempted by the establishment of corticostriatal circuits. An example of this in vitro models was achieved by Virlogeux et al., 2018 [107], with the reconstitution of a HD corticostriatal network-on-a-chip to establish synapses between axons of cortical neurons and dendrites of striatal synapses (Fig. 6D). The authors observed significant defects in the HD corticostriatal circuit compared to the wild type, by using a microfluidic device with high spatial and temporal resolution imaging. The authors found that a decrease in synapses and fire rate was prevalent in the cortical neurons rather than striatal neurons, which highlighted the important role played by the cortical compartment in the pathogenesis of striatal symptoms. These modeling in vitro platforms show their usefulness in deciphering the pathophysiology mechanisms of NDs and their potential to validate drugs and NFs of therapeutic and diagnostic interest.

Integration of biosensing systems in BoCs

Generally, the response of the in vitro organ models is often post-analyzed offline, which, besides being time-consuming, is prone to contamination and sample degradation, hampering the system's feasibility as a standard, autonomous and robust preclinical tool for laboratory practice [28]. Therefore, there is a demand for the integration of multiplexed (bio)sensors in OoC and, particularly, in BoCs, which enables the real-time monitoring of the physical environment of the culturing device, as well as the relevant brain's biomarkers that allow evaluating cell viability and toxicological or neuroprotective effects of drugs (or nanocarriers) for long periods. The possibility to integrate those (bio)sensors in the advanced microphysiological platforms for real-time and continuous

monitoring allows the creation of in vitro drug screening tools that confer robustness, continuous, and real-time data acquisition that other methodologies are unable to provide. Besides that, integration of sensors in BoCs allows high detection capability, high sensitivity, and minimal invasiveness of the biomodels [108]. Currently, and although, BoC platforms are evolving with extraordinary features to replicate the microenvironment of the human brain, namely: (i) new fabrication methods for the chips, (ii) novel hydrogel-based materials for the mimicking of ECM, (iii) recreation of the human brain physiology and functionality with human stem cells (i.e., neural stem cells, induced stem cells and embryonic stem cells) that can differentiate into many neural cells to reconstruct the human brain as a dynamic model on the molecular, cellular and structural levels; the generality of the current BoCs have limited ability to monitor biomarkers and physicochemical parameters. Thus, the integration of biosensors, which are devices capable of converting biological or biochemical reaction into measurable signals, can provide BoCs with the capability to perform automated assays with accurate quantitative analyses for their clinical translation [109]. The importance of this topic was the focus of a recent review article published by Cecen and colleagues, 2023 [109], which discussed the importance of the integration of biosensors in BoCs for their progress and clinical translation. In this review, the authors gather a plethora of studies where different type of (bio)sensors are being developed for integration in BoCs or lab-on-a-chip systems to monitor the chip culture environment (such as O_2, temperature and pH), cell activity, cell function, BBB integrity and response to external stimulation factors, including electrical, mechanical and drugs. These biosensors include electrical, optical and electrochemical. Among electrical biosensors, the TEER sensor has been the main in situ detection method successfully incorporated in BBB models and one of the most used to measure the barrier integrity and permeability in a noninvasive way, measuring the electrical impedance across endothelial layer [110]. The integration of this sensor in a BoC has elevated interest in the evaluation of BBB permeability and integrity to surrogate in vivo studies, the effect of shear stress in the barrier, to evaluate brain-targeting drug screening tests or the effect of inflammatory in stimuli conditions [111]. Currently, some BBB-on-a-chip are commercialized with integrated TEER readouts, such as the one provided by Mimetas Co., named as Organoplate, which allows the measurement up to 40 samples in less than a minute to evaluate the barrier permeability [112]. Multielectrode arrays (MEAs) are another electric sensor that enables noninvasive, high-speed recording and network mapping of extracellular electric field potentials [113]. However, conventional in vitro MEA, primarily utilize planar electrode interfaces, originally designed for monolayer cultures, which restricts the contact surface area with 3D organoids. In BoC, or 3D-brain organoids, the measurement of the electrical activity is also based on the existing planar MEA developed for the 2D neuron monolayers, which have advantages for the understanding of single neuron-neuron interaction and synaptic mechanism. Yet, they are not suitable for the 3D structure of cerebral models, since the simplified biofeedback recorded with the MEA is based on the measurement of the cell layer surface, representing a partial part of the organoid activity that has difficulties of reproducibility, maybe due to the disregard of the high electrical activity in the inner layers of the organoids. Thus, recording from these surfaces is inconsistent, as most active neurons are deeper within the organoid, making systematic assessment of their electrical activity challenging [114]. To overcome this technological limitation, in the recent years some works have been published dealing with the development of modified nanostructures, such as curved and folded shapes for MEA recording, including buckled, cylindrical, and shells [113], with the intent to wrap the brain organoid. Example of this feature is the work of Huang et al. 2022 [113], which proposed a 3D shell MEA composed of self-folding polymer leaflets with conductive polymer–coated metal electrodes, reporting great potential for high signal-to-noise ratio and 3D spatiotemporal brain organoid recording. Although in this design the organoid model is fully covered, the inner part of the brain model is not assessed. To surpass this limitation, a recent study was published by Phouphetlinthong and co-workers, 2023 [114], where a protruding cantilever microelectrode array was developed to monitor the inner electrical activity of cerebral organoids. In this work the authors report a new microfabrication process that enables the creation of protruding cantilever MEAs on beams, that rise vertically over two hundred microns, by utilizing the relaxation of internal stresses in materials deposited over a sacrificial layer. The authors report that cantilever MEAs can measure action potentials with strong signals, while maintaining a transparent substrate, enabling fluorescence analyses like calcium imaging. The improvement of 3D MEAs technology for the rapid growing field of BoC devices, will allow a deeply understanding of the organoid behavior and responses to various modifiable stimuli, enabling exploration and understanding of the brain, functionality and neuropathogenesis.

Other type of (bio)sensor is the electrochemical (EC), which can be used to measure pH, O_2, metabolic molecules or biomarkers. According to the EC reaction type can be classified as potentiometric, voltametric and amperometric. Although the integration of (bio)sensors in BoCs is a highly desirable technological feature with promising potentiality, there are few examples in

literature where in situ sensing analysis was successfully achieved. One of these examples is the pioneering work of Yu et al., 2019 [115], where an automated digital microfluidic (DMF) platform integrating dopaminergic cells with electrochemical analysis of dopamine homeostasis was developed. The presented platform was designed to examine the response of the dopaminergic cells (differentiates SH-SY5Y cells) after exposure to 4-dopamine transporter ant/agonists and study their pharmacokinetics. The innovative platform is claimed by the authors as the first integrated system able to perform real-time and continuous monitoring of dopamine uptake and release, highlighting the potential for future multiplexing that can be useful for screening libraries of drugs and/or NFs for neurotransmitter homeostasis.

Another sensing strategy with high potential for in situ monitoring of BoCs is based on optical biosensors, which convert different features of light (i.e., resonance, reflection, polarization and plasmon effect) into an electric signal due to the photoelectric effect [109, 116]. A good example of the capability of an optical sensor integrated into a BoC was recently described by Su and co-workers, 2023 [117], developed to monitor cytokine secreted by BBB. In this study, the authors integrated biosensors to cytokine profiling of luminal (blood) and abluminal (brain) levels of IL-6, MCP1(CCL2) and KC(CXCL1) using an ultrafast digital multiplexed immunosensor, called as DigiTACK platform, with short incubation/detection time (lower than 15 min) and very low limit of detection (LOD aprox. 100–500 fg/mL). This novel technological BoC sensing platform further emphasizes the potential of these microfluidic and sensorized bioplatforms for drug screening, as well as to better understand human physiology, diseases' mechanisms and new methods of pharmacological treatment.

Another exciting technological tool that promises to revolutionize neuroscientific studies is the integration of optogenetic techniques and computer automation with BoCs, allowing relevant data collection and image processing of the in vitro brain models [118]. The coupling of optogenetic and BoCs can be a strategy to gain a relevant and deeper understanding of the human brain and neurological diseases, using 3D in vitro models in a free-animal testing approach. Relevant reviews of optogenetics and its technological abilities can be found elsewhere [119, 120].

However, the development and integration of these new techniques into BoCs raises other challenges, such as the generation of a high amount of complex *omic* data (i.e., genomic, proteomic, metabolomic, pharmacogenomic, etc.), that needs to be processed and analyzed to extract hidden patterns. These compiled data can then generate relevant databases with privileged information to develop new medicines, novel therapeutic applications, and neurological discoveries. For that, artificial intelligence (AI) and machine learning algorithms can be the cornerstone tools to solve this problem [121, 122]. Examples of the applicability of AI for neuroscience are extensive, and go from the generation of complex deep neural network architectures [123], to the discovery of new neurological drugs [124], and gene targets [125]. Nevertheless, the application of AI in BoCs is not yet established, possibly due to the need of a consolidated multidisciplinary effort among various interdisciplinary teams.

Concluding remarks, future perspectives and challenges

BoC platforms have been developed with the potential to be applied as in vitro preclinical tools, replacing animal tests for the screening of drugs and/or nanocarriers, pathogenesis studies and advance of personalized medicine. Among the advantages, BoCs allow the decoupling of the complex and multi-structured brain into their counterparts to study specific cell-cell interactions, tissue microenvironment, or combine different sub-unit brain systems, such as the case of the vascular endothelial barrier (BBB) and brain (CNS) to study the BBB-crossing and brain-targeting of drugs/NFs. Another advantage is the ability to incorporate in situ (bio)sensors for continuous or real-time monitoring of biochemical reactions to external and/or internal stimuli (i.e., pH, O_2/CO_2, shear stress, diseases biomarkers, neurotransmitters, drugs, among others). On this regard, some studies have been developed with the purpose to generate autonomous BoC platforms with sensing integration, to serve as end-use drug screening platforms. In this perspective, it is expected that BoCs combined with multiplexed (bio) sensors can fulfil the existing technological gap for representative in vitro brain models dedicated to standard laboratory practice [28]. For that, AI and machine learning can be a needed aiding tool to gather and pattern information generated by those multiplexed (bio)sensors, creating robust databases and libraries for neurological new discoveries and drug developments, including advances in nanomedicine and neuroscience. It is then plausible that the fast advance of AI and machine-based learning tools for the treatment of big data, can be integrated in the future with multiplexed sensing BoC systems, leading to a new kind of cutting-edge microphysiological devices; perhaps even with advanced functionalities such as cognition and self-awareness, giving birth to a new scientific revolution. Yet, to achieve this disruptive technology standardized as the preferable preclinical methodology over the traditional animal models, some current challenges must be surpassed in the future. These primary challenges can be categorized into two groups: biological and technical [28]. The predominate biological limitation is the development of a universal cell culture media

with a suitable composition of nutrients and growth factors, similar with blood, that satisfy multiple cell types in co-cultured organ models [28]. Currently, this challenge is overcome by mixing cell culture media of the specific cells in equal parts. But when multiple organs are intended to be emulated in a HoC device, or in complex organs such as the brain, this challenge is further compounded [126]. Also, the continuous search for the optimized emulation of the physiological processes and interconnectivity between organ models, increase complexity with the need to introduce vascularization, organ scaling, control over cell density and immunological response [28]. On the technical challenges, the establish of standardized materials and fabrication techniques across different research laboratories is a bottleneck that hinders reproductible inter-experiments, large-scale and cost-effective production [126]. This achievement could lead to the promotion of collaborations and establish of OoC technology, including BoC, as the preferred adopted preclinical methodology. Despite these obstacles, BoC technology continues to progress rapidly, offering promising prospects for further advancement. Most importantly, the dynamic evolution of BoC technology harmonizes well with shifting regulatory paradigms, as demonstrated by the progressive stance of the US Food and Drug Administration, moving away from obligatory animal testing towards more sophisticated and ethical methodologies [127].

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Author contributions
R.O.R. concepted and wrote the main manuscript text. S-R.S and M.B-L, have revised the main manuscript text, supervising the writing process and content. All authors have approved the submitted version and have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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Data availability
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Competing interests
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