Supplementary Material

Recent progress in translational engineered *in vitro* models of the central nervous system

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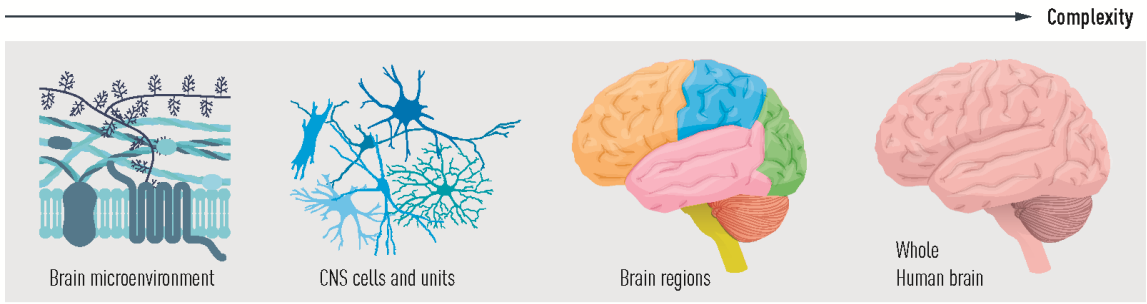
Supplementary Figure 1

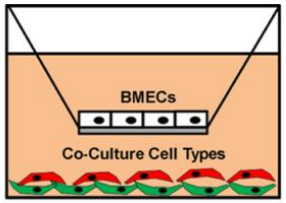
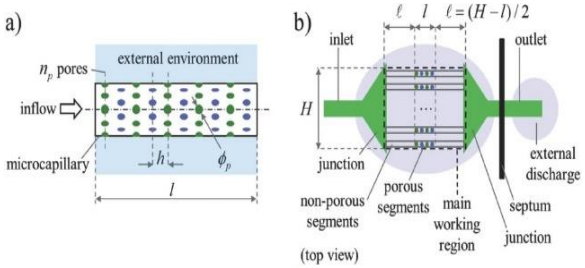
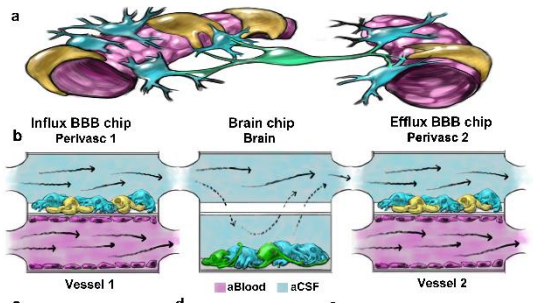
A

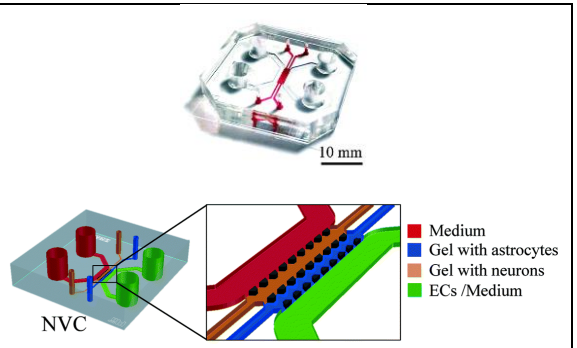


B

		Human relevance	Disease models	Systemic effect	Brain regions	Behavior	Electro-physiology	Mechanistic studies	ADME/ TOX	HTS	Cost
Standard 2D cultures	Human primary	+++	++	–	++	–	++	++	+	+	++
	Human iPSC	+++	+++	–	++	–	++	+++	+	+++	+++
	Rodent primary	–	++	–	+++	–	+++	+++	+	+	++
	Cell lines	+	+	–	–	–	+	+++	+	+++	++
Organoids	Human primary	+++	+++	–	++	–	–	++	–	–	++
	Human iPSC	+++	+++	–	+	–	+	++	+	++	++
	Rodent primary	–	+	–	+++	–	+	++	–	–	++
	Cell lines	+	+	–	–	–	+	++	–	++	+
Organs on a chip	Human primary	+++	++	++	++	–	++	++	++	–	++
	Human iPSC	+++	+++	++	++	–	++	+++	++	–	++
	Rodent primary	–	++	++	+++	–	+++	+++	+	–	+
	Cell lines	+	+	++	–	–	+	+++	++	–	+
	Rodent in vivo	–	++	+++	+++	+++	++	+	++	–	+++

Supplementary Figure 1: **Modeling the complexity of the human CNS**. A) The neural tissue is characterized by an immense cytoarchitectural complexity, illustrated in levels from left to right. The unique brain microenvironment, the vivid interplay among specialized neural cells, and the distinct regional characteristics are instrumental for brain functionality in health and disease. B) In CNS research, when attempting to create translatable models of the human brain, it is critical to reproduce the brain's unique functions, regions, and pathophysiology. Here, we have made an overviewing comparison of rodent *in vivo* models (the most commonly used mammal), standard two-dimensional (2D) cell culture models, organoid cultures and Organs-on-a-Chip (OoC) for their human specificity and their capacity to model human diseases, systemic effects, brain regionality, behavior, drug absorption, distribution, metabolism and excretion, and toxicity (ADME-Tox). We also rate the possibility for electrophysiological studies, detailed mechanistic studies, high throughput studies (HTS), and the cost of the model. For the three *in vitro* models, we divided them into the accessible cell sources, human primary cells, rodent primary cell and hiPCS, and cell lines. Notably, we want to emphasize that human primary cells from the CNS are scares. We further wish to highlight that this rating, the appropriateness of each model, varies for each specific study, and our rating should be used as a general guideline of what is possible to achieve with each model.

Model	Refs	Shear stress	Cell-cell interactions	High through put / Cost	Similarity to human physiology	Graphical representation[1]
TW [2]	(Zenker *et al.*, 2003; Colgan *et al.*, 2008; Helms *et al.*, 2014; Labus *et al.*, 2014; Canfield *et al.*, 2017; Delsing *et al.*, 2018)	No	Co-culturing possible, tri-culturing more challenging to evaluate cell populations	Yes / Low	Minimal, ECM present only as anchoring points, 2D geometry	
Porous-tube models	(Neuhaus *et al.*, 2006; Cucullo *et al.*, 2008; Marino *et al.*, 2018; Moya *et al.*, 2020)	Yes	Same as TW	Minimal / Moderate	Improved similarity to human physiology (shear stress, 3D lumical geometry), but minimal ECM present	
Microfluidic chips (membrane-based) [3]	(Booth and Kim, 2012; Prabhakarpandian *et al.*, 2013; Achyuta *et al.*, 2013; Wang *et al.*, 2017; Maoz *et al.*, 2018)	Yes	Capability of compartmentalization and studying interactions between cell populations	Yes, however more time consuming than TW / Moderate	Same as porous-tube models	

Microfluidic chips (ECM-based)	(Brown *et al.*, 2015; Herland *et al.*, 2016, Xu *et al.*, 2016*a*; Adriani *et al.*, 2017; Partyka *et al.*, 2017)	Yes	Same as membrane-based microfluidic chips	Yes, however more time consuming than TW / Moderate	Utmost attmept at in vitro biomimicry (shear stress,3D geometry, ECM present)	

Supplementary Figure 2: **Summary of *in vitro* models commonly used in BBB research**

TW: Transwell; ECM: extracellular matrix; 2D: 2 dimensions; 3D: 3 dimensions; BMECs: brain microvasculature endothelial cells; PDMS: Polydimethylsiloxane; NVC: Neurovascular chip

Porous-tube models

a. Graphical representation of a microcapillary-mimicking porouts tube that enables exchange with the external enviroment

b. Top view of porous tubes, many tubes can run in paraller.

Microfluidic chips (membrane-based)

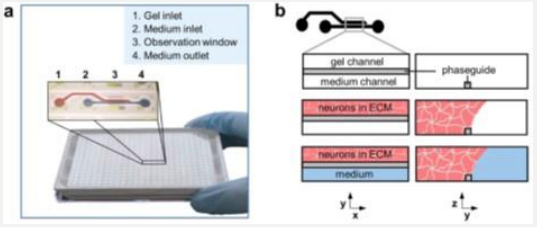
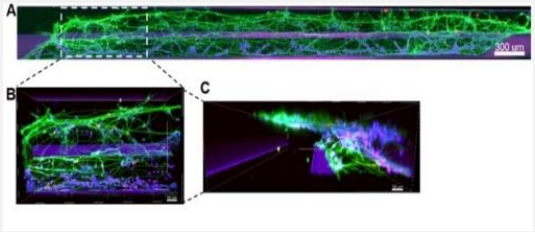
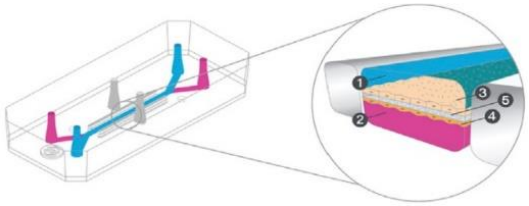
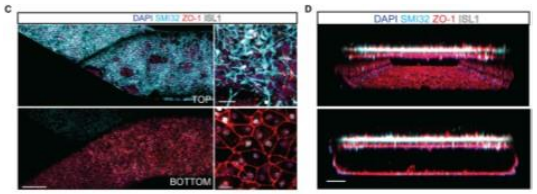
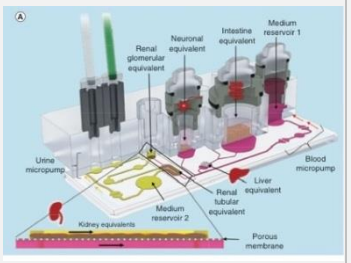
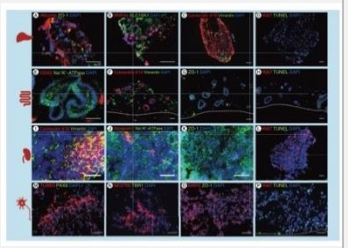
a. A simplified graphical representation of the NVU

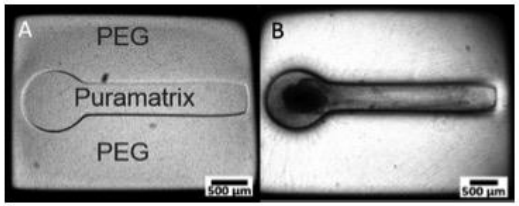
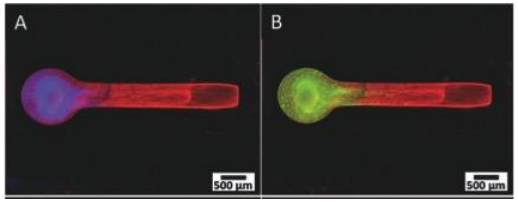
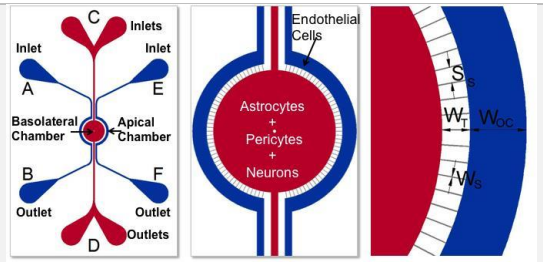
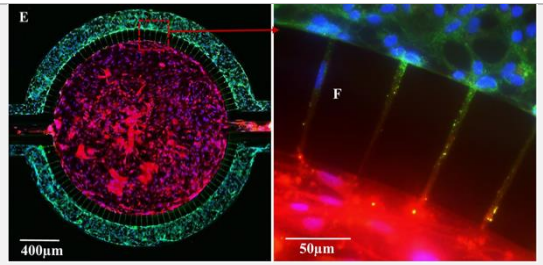
b. A linked NVU-on-Chip. hBMECs (magenta) are cultured with brain astrocytes (blue) and pericytes (yellow) in the top compartment of the chips; human brain neuronal cells (green) and astrocytes (blue) are cultured in the lower compartment.

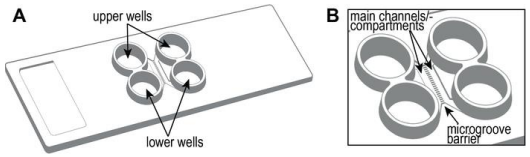
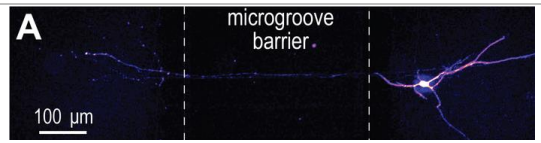
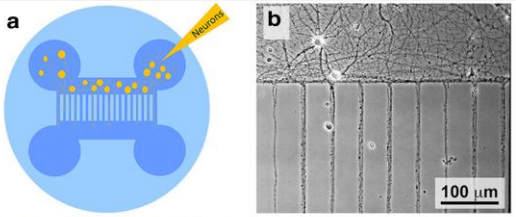
[1]TW model reprinted with permission from (Canfield *et al.*, 2017); microfluidic chip model (membrane-based) reprinted with reuse permission from the original work of the corresponding authors from (Maoz *et al.*, 2018); porous-tube model model reprinted with permission from (Marino *et al.*, 2018); microfluidic model (ECM-based) reprinted with permission from (Adriani *et al.*, 2017);

[2] In this list, we consider studies that use TW in static cultures, there are, however, studies that implement flow in TW (Hinkel *et al.*, 2019).

[3] In this list, microfluidic chips with a temporary membrane (i.e. a membrane that degrades over time) are not included, such as the work of (Tibbe *et al.*, 2018).

Developer	Engineered Devices	Application Examples	Strength and Limitations
MIMETAS the organ-on-a-chip company	 In (a) Schematic representation of an OrganoPlate, made of 96 microfluidic chips. In (b) experimental sketch for culturing 3D neuronal-glial networks mixed with ECM (From Wevers et al., 2016)	 Confocal reconstructions of stem cells derived dopaminergic neurons grown inside the OrganoPlate device (from Kane et al., 2019).	Cell can be embedded within the hydrogel, resembling the parenchymal space Cells can be coated on the hydrogel surface to mimic the vascular interface Versatile Possibility to grow 3D-culture system Highly compact and higher-throughput than competitors Only operational with bi-directional flow
emulate	 Schematic representation of dual-channel microfluidic chip. In the cross-section note the possibility to grow distinct cultures, separated by a porous membrane (from Sances et al., 2018).	 Confocal reconstructions of iPSCs derived motor neurons co-cultured with brain microvascular endothelial cells (from Sances et al., 2018).	Presence of two microfluidic channels with the possibility to culture two different types of cells Possibility to modulate and mimic various tissue specific fluid conditions Made of PDMS Cell-to-liquid ratio Surface-to-volume ratio Thickness of the membrane does not match the thickness of the basement membrane Not easily adapted to high-throughput assays
TISSUSE Emulating Human Biology	The microfluidic four-organ-chip, designed to host intestinal, liver, renal and brain cultures, mimicking the in vivo situation (from Ramme et al., 2019). 	Immunostaining of the induced plutipotent stem cell-derived liver, intestinal, renal and neuronal cultures grown in the four-organ chip (from Ramme et al., 2019). 	Built-in micropump driven by an external pneumatic controller Constructed of thermoplastic while PDMS is restricted to a thin membrane Open tissue chamber and separated from the fluid channels Possibility to combine with different tissue assembly method Tissue volume scaling Cell-to-liquid ratio

			Not easily adapted to high-throughput assays
AxoSim Human Data, Faster.	 The microengineered nerve-on-a-chip device enabling the growth of parallel neural fibers bundles for physiological testing (from Huval et al., 2015).	 Fluorescent reconstructions of neuronal fibers growth and migration immunostained for β-tubulin III (red) and glial cells (green). Nuclei are visualized by DAPI (blue) (from Huval et al., 2015).	Ideal for clinical nerve compound action potential (CAP) and nerve fiber density (NFD) tests 3D in vitro system Succesfully adapted for electrophysiological recordings Only tested on rat tissue explants Not easily adapted to high-throughput assays
SYNVIVO	 Scheme of the BBB model, displying the apical chamber (vascular tissue) and the basolateral chamber (brain tissue cells). Porous architecture enables communication between the vascular and brain tissue).	 CD-31 (green) stained endothelial cells and GFAP (red) stained astrocytes forming tight cells junctions in the SynBBB device (from Deosarkar et al., 2015)	Possibility to maintain and image the micro-vessel for long periods of time Tissue compartment and microvascular channels that mimic the 3D-morphology of in vivo microvessels Porous interface that replace the use of membranes Size of the micro-channels Not easily adapted to high-throughput assays

XONA MICROFLUIDICS	 Sketch of the multicompartment microfluidic chip, showing the channels and the microgrooves seprating the channels (from Paranjape et al., 2019)	 Neuron extending axons trhough the microgrooves of the chip and into an isolated axon compartment (from Paranjape et al., 2019)	Made of cyclic olefin copolymer No autofluorescent Ideal hydrophilic surface for attachment and growth of stem cells Possibility of co-cultures Gas impermeable Not easily adapted to high-throughput assays
ananda	 The Neuro Device used to pattern neurons and direct axonal extension. It enables growth of 100 axons with more than 1 mm in length (from Magdesian et al., 2016).	 Neurons cultured in the PDMS Neuro Device, enabling the growth of axons and dendrites inside the microchannels (from Magdesian et al., 2016).	Possibility to be removed any time for direct manipulation of neurons Good for axonal extension measurements Possibility of co-cultures Made of PDMS Not easily adapted to high-throughput assays

Supplementary Figure 3: **Commercial OoC or chip providers.** Overview on commercial microfluidic chip providers, with a description and application of their device for brain application studies.