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Review article

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Functional bioengineered models of the central nervous system

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Abstract

The functional complexity of the central nervous system (CNS) is unparalleled in living organisms. Its nested cells, circuits and networks encode memories, move bodies and generate experiences. Neural tissues can be engineered to assemble model systems that recapitulate essential features of the CNS and to investigate neurodevelopment, delineate pathophysiology, improve regeneration and accelerate drug discovery. In this Review, we discuss essential structure–function relationships of the CNS and examine materials and design considerations, including composition, scale, complexity and maturation, of cell biology-based and engineering-based CNS models. We highlight region-specific CNS models that can emulate functions of the cerebral cortex, hippocampus, spinal cord, neural-X interfaces and other regions, and investigate a range of applications for CNS models, including fundamental and clinical research. We conclude with an outlook to future possibilities of CNS models, highlighting the engineering challenges that remain to be overcome.

Sections

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Key points

- Isolating the mechanisms of central nervous system (CNS) functions will benefit human health and well-being, decrease economic burdens and inspire the design of neuromorphic computers and artificial intelligences.
- Customizable, bioengineered tissues can recapitulate CNS structures and functions, representing simplified platforms that allow the systematic assessment of neural development and pathology in vitro.
- CNS regions, such as the cerebral cortex, hippocampus, brainstem and spinal cord, can be modelled with organoids, spheroids, microfluidic chips and bioprinted or scaffold-based constructs that combine cells and materials.
- Increasingly biomimetic CNS models have the potential to display higher-order functions, including cognitive abilities and conscious experience.

Introduction

A functional understanding of the central nervous system (CNS) lies at the core of some of the biggest unanswered questions in science, such as the nature of consciousness[1] or the boundaries of life and death[2], because brains encode the unique patterns of thought, feeling and behaviour that define the individual. The socio-economic impact of our incomplete knowledge of CNS function is best illustrated by the rising annual worldwide costs associated with Alzheimer disease, which are estimated to have exceeded $1 trillion per year in 2020 and are expected to reach US$9 trillion per year by 2050 (ref. [3]). Nevertheless, known principles of CNS function are inspiring the design of artificial intelligences, which have become increasingly enmeshed with day-to-day human life[4]. However, new techniques must be developed to achieve a comprehensive understanding of the CNS.

Animals were long used as models of human anatomy, physiology and behaviour[5], and have contributed to key discoveries in CNS biology, including the characterization of the action potential[6]. Their ubiquity in biomedical research is a testament to their predictive validity[7]; however, to improve physiological relevance and translational outcomes, and to increase tractability, human-based CNS models are required. In addition, animal housing and handling costs, facility requirements, low sample sizes and ethical concerns have, in many cases, made in vitro models more appealing to understand CNS function. Although CNS tissues of animals closely reflect those of humans, they are inflexible experimental tools; that is, animal brains are constrained by morphogenetic factors that determine a highly restricted range of phenotypes. We have gained a wealth of knowledge by probing conserved nuclei and tract systems in animal models, but many fundamental questions require greater degrees of control and are, therefore, impractical to answer in vivo.

Innovations in stem cell biology and neural tissue engineering have made the bioengineering of customizable, 3D in vitro CNS models possible, enabling the study of human neural development and disease (Fig. 1). Soon after the development of the first techniques to visualize microscopic brain anatomy[8], basic colloidal hydrogels were developed[9] and CNS tissues were cultured for the first time with frog cells using 'the hanging drop' technique[10], followed by chick and rodent cell culture in flasks[11]. The identification of multipotent stem cells[12] and their presence in the brains of postnatal rats[12] then enabled the design and control of bioengineered tissues using hydrogels based on cross-linked polymeric networks that would serve as tissue scaffolds and cell-infused inks[13]. Developments in microelectrode array (MEA) technology[14,15], bioprinters[16] and microfluidic devices[17,18] allowed the construction and functional assessment of bioengineered CNS models with high precision. Today, neural cells can be created by reprogramming induced pluripotent stem (iPS) cells from fibroblasts[19,20], enabling the creation of cerebral organoids[21] with region-specific identities[22] and complex functions[23]. Similar functional capacities can be achieved in scaffold-based 3D neural tissues[24,25]. In addition, the blood–brain barrier (BBB) can be modelled in microfluidic devices[18] and bioprinted constructs[26] (Fig. 1). Such functional in vitro models of the human CNS must recapitulate some, but not all, features of the CNS. Bioengineered tissues typically lack some native elements of CNS tissues and, thus, may not capture recondite interactions. However, they should be sufficiently biologically representative to address a specific research question (Box 1).

3D bioengineered neural tissues are becoming increasingly inexpensive, reproducible, scalable and susceptible to high-throughput investigations[27]. They also benefit from higher complexity compared with monolayer cultures, recapitulating neural microenvironments without the intrinsic damage associated with the preparation of organotypic slice cultures[28]. Importantly, bioengineered CNS models can be precisely configured, in which cell type and density, extracellular matrix (ECM) composition, orientation, geometry, mechanical properties and interfacial properties can be customized to test specific hypotheses or to enable transplantation.

Structure and function of the CNS

Modelling the CNS requires an understanding of its basic structure–function relationships at multiple scales (Fig. 2). At the macroscale, the CNS is a collection of heterogeneous tissues encased within the bones of the skull and vertebral column. Their coordinated activities enable sensation, perception, memory and voluntary movement. Each neural region's specialized function is determined by its tissue cytoarchitecture, which is defined by cell-type composition, neural microenvironment and local circuit structures, as well as the configuration of efferent and afferent connections that link it with other regions within and outside the CNS.

The cerebrum comprises several mesoscale tissues, including the cerebral cortex, the hippocampus and several basal nuclei, or ganglia, which provide executive control[29], memory encoding[30] and conscious experience[31]. Deep within the centre of the cerebrum are the thalamic and hypothalamic nuclei, which are collections of cells that transmit signals from the brainstem to the cortex[32] and regulate autonomic functions[33], respectively. Anatomically continuous with the thalamus are the divisions of the brainstem: the midbrain, pons and medulla. Each subdivision contains nuclei with distinct sensory, motor and vital functions. Functionally coupled to the brainstem is the cerebellum that coordinates the timing and execution of fine movements as well as features of cognition[34]. The spinal cord, which is continuous with the brainstem and runs down the centre of the vertebral column, consists of a core of cell bodies surrounded by nerve fibres that receive sensory data from and transmit output motor sequences to the peripheral nervous system[35]. The CNS also contains specialized olfactory and retinal tissues, which transduce chemical and optical signals, respectively.

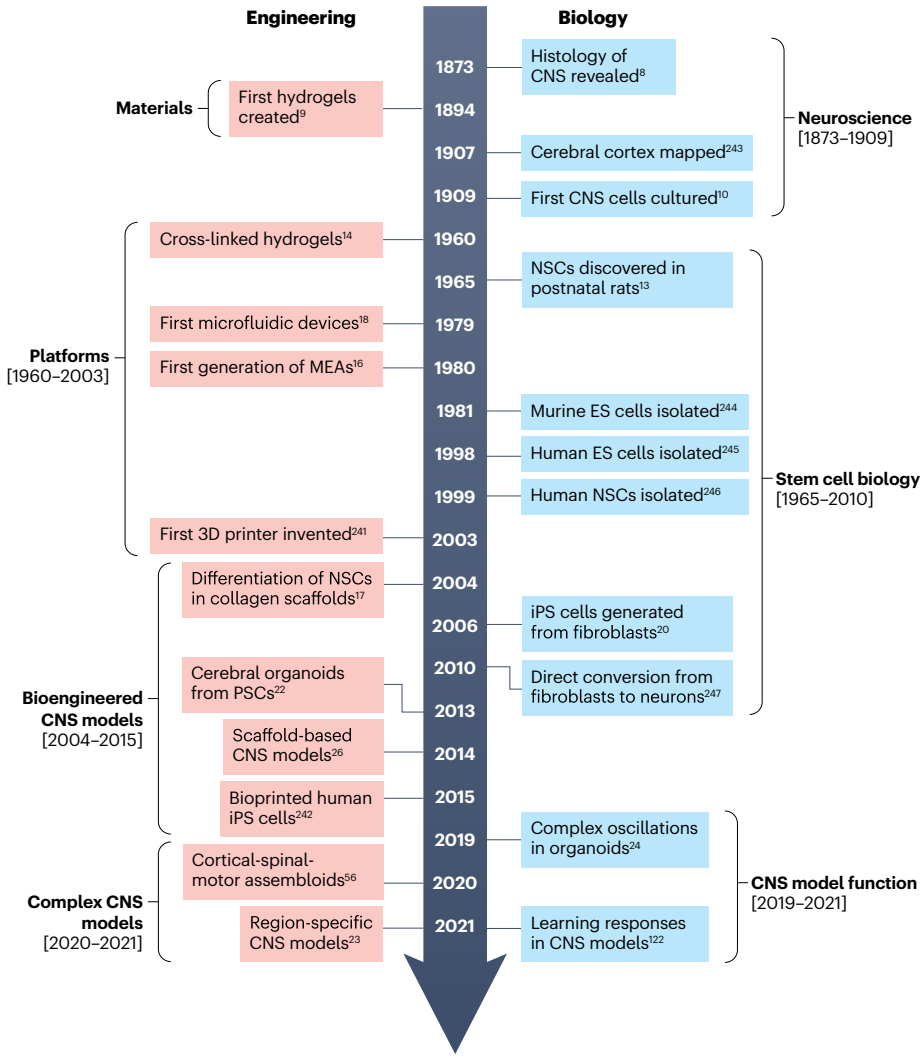

Fig. 1 | Timelines of engineering and biology advances towards neural tissue engineering. CNS, central nervous system; ES cell, embryonic stem cell; iPS cell, induced pluripotent stem cell; MEA, microelectrode array; NSC, neural stem cell; PSC, pluripotent stem cell[239–246].

At the microscale, the CNS is composed of several distinct cell populations. Neurons are electrically excitable cells that are specialized for rapid cell-to-cell communication. They synthesize neurotransmitters and neuropeptides which serve as information carriers within complex networks. Oligodendrocytes interact with neurons to form an insulating myelin sheath along neuronal axons, increasing signalling rates tenfold. Ependymal cells secrete cerebrospinal fluid to clear waste, maintain brain buoyancy and mitigate mechanical impacts. In addition, astrocytes buffer extracellular molecules, and microglia perform immune functions. These and other cells of the CNS exist cooperatively within a complex microenvironment composed of a proteoglycan-rich ECM[36] that is further specialized at neuronal surfaces by perineuronal nets[37], facilitating the stabilization of connections between cells within neural networks. CNS cells, such as pericytes and astroglia, also form specialized vascular interfaces that define the BBB[38]. If cells of the CNS are damaged or if their ability to release, transport or sequester specific signals is compromised, they can precipitate psychological states of depression, anxiety and psychosis[39].

Design considerations of CNS models

Bioengineered CNS models can capture only key features of native CNS tissues, and therefore several design elements should be considered to maximize their validity as experimental tools (Fig. 3).

Composition

Perhaps the most essential design consideration involves the selection of relevant cell types for co-culture. In addition to the many subtypes of glia and other non-neural cells, molecular analyses have revealed various neuronal subtypes in the human brain[40,41]; however, the true number likely ranges from several hundred to a thousand[42]. Primary tissue extracts from animals can provide balanced ratios of excitatory and inhibitory neuronal populations and native glial cell concentrations; however, the presence or absence of any particular cell type in primary

Box 1

How complex is complex enough?

The process of understanding the brain can be described as a "journey from complexity to simplicity"[70]. Although highly detailed, nanoscale models of brain function might be technically achievable, impressive and generally predictive; they would ultimately fail to yield conceptually meaningful information about brain functions. Instead, reducing the brain's intrinsic complexity to simple, coarse-grained and hierarchical models with varying levels of biophysical detail may better enable the investigation of brain functions and the underlying mechanisms. As tissue engineering technologies continue to improve, allowing higher degrees of complexity, model systems may be engineered that are effectively too complex to inform a fundamental understanding of brain function.

Model systems are, by design, minimally representative versions of their natural templates that trade complexity for tractability. Indeed, by reducing the number of explanatory variables, models serve to isolate causal elements from their correlative counterparts, which would normally obscure mechanisms in vivo. Just as T. H. Huxley explained in the mid-nineteenth century, some organisms may serve as convenient models of others without the expectation that they "should be absolutely and precisely equivalent one to other"[247]. Thus, simple, bioengineered model systems that are approximately equivalent to their natural templates may be sufficient to investigate brain functions.

Organoids are complex tissues with internal cytoarchitectures that reflect native tissue organization; however, they are comparatively simple relative to in vivo tissues. Microfluidic devices reduce central nervous system (CNS) structure–function to its most distilled elements, promoting an understanding of the most basic interactions between defined cell types and their microenvironments.

Tuning complexity can also lead to new insights. To better understand neurodevelopment, it may be necessary to increase tissue complexity by incorporating spatio-temporal gradients of morphogens or by introducing non-neural tissue interfaces to recapitulate in vivo embryonic processes. To disentangle the functional roles of components of the blood–brain barrier (BBB), it may be required to iterate many simplified versions of the neurovascular unit, systematically knocking cell types in or out, and evaluating outcomes. These are just two examples within the greater multifactorial design space that is enabled by neural tissue engineering.

tissue isolates is dependent upon neurodevelopment[43]. Therefore, cells must be harvested from embryonic, perinatal or adult animals, depending on the purpose, balancing cell viability and plasticity with region-specific phenotypes.

The goal of CNS modelling is often the investigation of human physiology and disease, and therefore iPS cells, embryonic stem (ES) cells or neural stem cells (NSCs) from human sources should be included. These can be differentiated along specific neural lineages, generating programmed neuronal populations with specific neurotransmitter and neuropeptide profiles[44] as well as astrocytes, microglia and oligodendrocytes[45]. Patient-specific cells can also be harvested and transformed to CNS lineages to generate personalized human CNS models with the individual's genetic material to optimally simulate their unique disease phenotype and overcome treatment resistance with high-throughput drug screening[46]. Cells and species can be combined in vitro, including human–chimpanzee[47,48] and other hybrid systems. Indeed, evolutionary hypotheses can be addressed by inserting the genes of extinct hominids, such as Neanderthals, into human brain organoids[49].

In addition to cell-type composition, materials must be carefully selected to create scaffolds (for example, hydrogels), bioinks as well as other bulk components and surfaces to simulate distinct CNS microenvironments. Importantly, the elastic modulus of adult brain ECM (0.1–1.0 kPa) differs greatly from embryonic brain ECM (110 Pa) or spinal cord ECM (90–230 kPa)[50]. Biophysical cues modulate cell migration, differentiation and neurotransmission, and therefore ECM properties, such as stiffness, should be considered in the CNS model[51]. Mammalian polymers, such as collagen or hyaluronan, can be purified from decellularized ECM extracts to generate physiologically relevant hydrogels and scaffolds[52]. Alternatively, synthetic polymers provide flexible, biocompatible options with highly tunable properties[53].

Dimensions

Bioengineered CNS tissues can be designed sufficiently modular to support multiscale patterning, as observed in native tissues. For example, organoids can be fused to create 'assembloids' with circuit-like or system-like properties[54], including neuromuscular junctions[55]. Neurospheroids, formed by microscale cell aggregations, can be daisy-chained into functional arrays, or shaped by polydimethylsiloxane (PDMS) moulds into mesoscale tissue blocks, which can, in turn, be combined to form macroscale networks of neural modules[56]. Bioprinting supports customizable tissue geometry with single-cell precision[57], and laminar organizations characteristic of the cortex[58] or neurovascular unit[26]. Scaffolds offer optimal control over compartmentalization of tissue types; for example, toroidal geometries provide high surface areas and centre surround-type organizations that reflect the nested nuclei of CNS regions, such as the amygdala, hippocampus and hypothalamus. In addition, microfluidic conduits or etched surfaces can be engineered to guide neurites or vasculature, and to polarize tissue structure[59].

Maturity

Cell maturity is an important element in CNS modelling, because plasticity, regenerative potential, genetic expression, metabolic activity and disease processes are dependent upon development. Models derived from human stem cells and non-human embryonic primary tissues rich in progenitors may be ideal tools to investigate neurodevelopmental disorders (for example, autism, schizophrenia)[60]; however, such models may not be representative of adult phenotypes, which are less plastic, more specialized and have less regenerative potential compared with embryonic tissue-based models. The ageing methylome — the total distribution of cytosine-bound methyl groups in the genome — and its epigenetic interactions with genotypic differences of sex and ethnicity

should also be considered[61]. Cells with high stemness may extend cell viability for long-term culture, but are less relevant as models of CNS injury or degeneration[62]. Tissue maturity can also be tuned independent of cell sources by changing the mechanical properties of scaffolds; here, material stiffness can mimic ECM alterations associated with normal ageing[63] and age-related disease[64]. For example, the expression of a truncated form of lamin A protein (progerin) induces ageing and disease phenotypes in a genetically susceptible iPS cell-based model of Parkinson disease[65]. In addition, pH[66], electrical conductivity[67], viscoelasticity[68] and physiological concentrations of iron and other metals[69] may be customized to achieve phenotypes that reflect different states of brain maturity.

Complexity

The CNS is the most functionally complex organ in the human body. Complexity describes the interaction of a set of elements to generate non-linear, synergistic or emergent functions that are greater than the sum of their parts. As elements are removed from a system to evaluate their roles, fewer interactions are possible and complexity may decrease. Therefore, modelling the CNS should strike a balance between functional relevance and utility (Box 1). Bioengineered models of the CNS are often deliberately designed to be less complex than their natural templates because simple models are more tractable than in vivo equivalents, offering greater control over each element and the number of potential interactions (for example, neural, endothelial, glial, ECM)[70]. From an experimental perspective, fewer possible interactions increase the likelihood of identifying causal factors that contribute to development, regeneration, injury or disease. However, the elimination of key elements can contribute to the suppression of emergent or synergistic functions and, therefore, to spurious conclusions. Thus, complexity must be considered at scale, depending whether the research question may be better addressed by low-complexity models that simulate simple circuits[71] or high-complexity alternatives that simulate networks and systems[72,73].

Interfaces

The brain and spinal cord interface with multiple tissues to orchestrate bodily functions. Cranial nerves and ascending tract systems from the spinal cord relay sensory data to the brain, informing cognitive functions. Other cranial nerves and descending tracts conduct information from the brain to lower-level effectors that drive muscle activation to enable ambulation and speech. Without sensory inputs and motor outputs, functional models of the CNS are closed-loop systems that are intrinsically resistant to assessment of higher-order functions. Similarly, the integrity of the CNS is dependent upon the perfusion of nutrients and oxygen across neurovascular interfaces at single-cell resolution. Moreover, systems-level inputs from the cerebral ventricles, the gut and other viscera are important.

Modelling the CNS

Modelling approaches

Functional CNS models can be designed by engineering-based or cell biology-based approaches (Fig. 4). Cell biology-based models, which include organoids, spheroids and assembloids, are generated by cell-autonomous processes, resulting in the self-organization of tissues into complex architectures consistent with the development of organisms[28]. Despite their unparalleled recapitulation of native CNS structure–function, cell biology-based models lack the intrinsic tunability of engineering-based models that combine cells with materials to direct tissue patterning. Here, tissue organization can be engineered by bottom-up approaches, that is, organizing modular tissues into complex structures, or top-down approaches, in which cells are seeded within pre-assembled 3D microenvironments that provide cues for self-organization[74].

Organoids. Neural organoids are formed by cellular self-organization with inputs from soluble factors and other microenvironmental cues. To generate organoids, embryoid bodies composed of aggregated pluripotent stem cells (PSCs) are typically embedded in Matrigel (solubilized native matrix rich in ECM components, such as laminin, collagen and embedded factors), in which cell sorting and tissue patterning progress towards a 3D morphology that is 0.5–4.0 mm wide with embryonic CNS features, ideally suited for studies of neurodevelopment and evolution[75]. Non-directed protocols, which lack factors to guide tissue patterning, yield whole-brain phenotypes termed cerebral organoids, which are highly variable in cell-type composition and spatial organization[28]. Dual SMAD inhibition can induce neural ectodermal fate commitment[22]. Alternatively, in directed protocols, additional morphogens are introduced to generate region-specific neural organoids[22] by guiding tissue growth and patterning.

Organoids have been developed with cortical, hippocampal, diencephalic, mesencephalic, ventricular and cerebellar phenotypes[22]; however, tissue organization and cell type ratios remain incomplete. In addition, the integration of 3D vasculature to support stable, long-term viability of organoids remains limited, often suffering from poor perfusion of nutrients and oxygen, which restricts organoid volume and can result in necrotic cores[76]. Moreover, organoids are not particularly modular structures and, similar to organisms, are limited by conserved developmental programmes.

Spheroids. Neurospheroids consist of aggregated cells from primary tissues, immortalized cell lines or stem cells. In the absence of adherent surfaces, or when forced together by centrifugation or gravity, cells adhere to each other[77], and therefore spheroid generation does not require scaffolding or other external support. Spheroids establish their own 3D microenvironments by secreting ECM[78]; however, their internal structures are less reflective of in vivo cytoarchitecture compared with organoids. Although spheroids are typically less than 1 mm in diameter, their cell densities (10^5–10^6 cells mm^{-3}) are similar to in vivo CNS tissues[56]. Therefore, despite their uniformity and simplicity, spheroids represent excellent models of neurodegenerative disease, brain tumours and other neuropathologies. To increase their scale and complexity, spheroids can be fused to form cuboid, mesoscale neural building blocks with cell-specific identities[56]. These, in turn, can be connected to generate macroscopic ensembles that display functional network properties. However, without microfluidic-assisted perfusion or other supports, spheroids lack vascularization, preventing continuous, long-term culture[79] and limiting their utility for studies of maturation, senescence or long-term exposure to drugs and environmental hazards.

3D-bioprinted models. Bioprinted CNS models combine materials and cells to form 3D tissue constructs with single-cell spatial resolution. In direct bioprinting, cells are infused with hydrogels to create printable bioinks that can be extruded as continuous filaments, ejected as discrete droplets or sculpted by ultra-precise, laser-based printing techniques to form complex 3D tissue architectures[26]. Printable materials include alginate, collagen, chitosan, gelatin, poly(ethylene glycol)

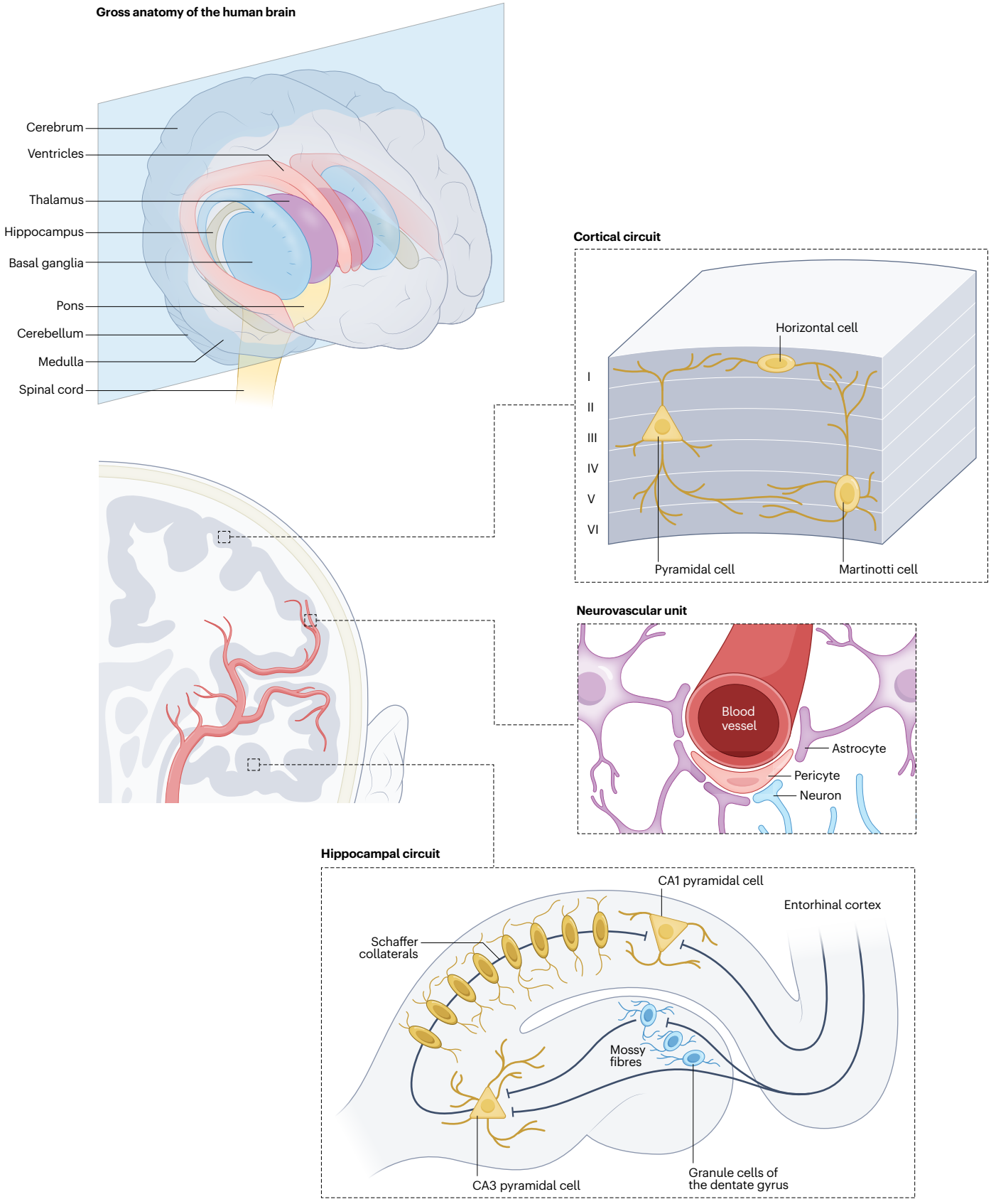
Gross anatomy of the human brain
Cerebrum
Ventricles
Thalamus
Hippocampus
Basal ganglia
Pons
Cerebellum
Medulla
Spinal cord
Cortical circuit
Horizontal cell
I
II
III
IV
V
VI
Pyramidal cell
Martinotti cell
Neurovascular unit
Blood vessel
Astrocyte
Pericyte
Neuron
Hippocampal circuit
CA1 pyramidal cell
Entorhinal cortex
Schaffer collaterals
Mossy fibres
CA3 pyramidal cell
Granule cells of the dentate gyrus

Fig. 2 | Anatomy of the central nervous system at multiple scales. Bioengineered tissue models of the central nervous system (CNS) are designed and built to mimic the structures and functions of in vivo tissues. At the macroscale, the CNS can be subdivided into gross regions, including the cerebrum, brainstem and spinal cord. The cerebrum can be further partitioned into several mesoscale sub-organs, such as the cerebral cortex, basal ganglia and hippocampus — all of which are encased within a dense field of nerve fibres (that is, white matter). At the microscale, cells form highly ordered circuits, interfaces and pathways that reflect complex functions. Cortical and hippocampal circuits display re-entrant or looped activation patterns, which enable cell synchronization and the integration of information associated with perception and memory. However, when left unchecked, re-entrant loops can precipitate seizure activity. Similarly, the neurovascular unit forms a blood–brain barrier (BBB) by tightly joining endothelial cells, pericytes, glial cells and neurons in concentric layers — a selectively permeable gateway that becomes compromised with neurodegenerative disease, cancers, infections and trauma. At finer scales, cells are immersed within complex microenvironments patterned with biophysical and biochemical cues that determine their fate and behaviour. To accurately model CNS function in vitro, the essential elements need to be identified that optimally recapitulate structures and functions of the CNS. CA, cornu ammonis.

diacrylate (PEGDA) and silk fibroin, which can be partially cross-linked or doped with catalysts prior to printing for rapid gelation[80]. Printing needles can be designed to enable simultaneous deposition of cells, gels and cross-linkers using co-axial nozzle configurations[81], thus allowing high spatial resolution and fabrication of hollow tube structures. Cells from primary tissues, immortalized lines, stem cell donors or combinations thereof can be incorporated in bioinks to customize the properties of the model system, including viability, plasticity or regenerative competence. However, 3D bioprinting is currently limited by material constraints (such as viscosity), shear forces, lower cell densities relative to native tissues and cell-perturbing gelation methods involving chemical and light-based catalysts.

Microfluidic models. Microfluidics-based CNS-on-a-chip models distil the most essential features of a physiological process from multivariate neural systems. Although less complex than other models, microfluidic-based CNS models allow the incorporation of biophysical and chemical signalling gradients to mimic microenvironmental cues, interstitial fluid flow and single-cell resolution neurite guidance[82]. Microfluidic PDMS platforms can be fabricated by soft lithography, using a template to emboss and print identical replicate chips for maximum reproducibility and high-throughput modelling[83]. These chips are optically clear, and thus CNS-on-a-chip models enable real-time monitoring of cell migration and network formation[84]. Moreover, specialized tissue–tissue interfaces can be designed in multichannel chips to model the BBB[17] and the gut–brain axis[85]. Although versatile tools for drug discovery, microfluidic-based models are not ideal implantables, which may be addressed by integrating materials with high biocompatibility, biodegradability and wireless controllers[86]. In addition, they often lack 3D cytoarchitecture, they contain only few cell types and PDMS can absorb drugs and proteins[87].

Materials

Engineering-based 3D modelling techniques combine cells with suitable materials to pattern tissues into reproducible 3D constructs. To guide their initial assembly and support long-term function, materials for CNS modelling[52] should reflect the physical, chemical and mechanical properties of native tissues and their unique 3D microenvironments. In addition to providing structural stability, materials can facilitate oxygen and nutrient transport, waste outflow and ECM deposition. Materials can be modified by altering local surface topology or, in bulk, by integrating cell signalling peptides to increase biocompatibility.

Matrix scaffolds and hydrogels. The in vivo conditions of the CNS can be closely mimicked by decellularized CNS tissues, including the meninges. These ECM scaffolds can be re-seeded with different cell populations[88], serving as signal-rich microenvironments for neural precursor cells and promoting viability, adherence and differentiation. Hydrogels composed of collagen, hyaluronic acid, silk fibroin, alginate or chitosan[28,36] also closely approximate natural ECM scaffolds[36], and their cross-linked polymeric network structures can be infused with biological factors to improve cell growth and proliferation, or to guide tissue organization. Alternatively, synthetic polymers, such as polyvinylidene fluoride, have piezoelectric properties that can guide cellular signalling and regeneration[89]. Similarly, poly(3,4-ethylenedixoythiophene) (PEDOT) layers can be integrated with hydrogels to enhance electrical conductivity[90]. Depending on the material, hydrogels can be made biocompatible, enabling cellular grafting to repair injured brain tissues following trauma or stroke[91]. Cell-free scaffolds, channels and hydrogels may also be fabricated prior to cell seeding by indirect bioprinting, extending the selection of materials and techniques to mimic 3D neural microenvironments[26]. However, such scaffolds suffer from batch-to-batch variability, size and density restrictions, and scaling issues, which can limit high-throughput applications[92].

Biomaterials. Cells of the CNS are embedded within and connected by a specialized ECM that maintains tissue stability, regulates diffusion of molecules, controls local biomechanical signalling, participates in synaptogenesis, guides neurite outgrowth, supports cell migration and inhibits tissue remodelling. The main components of brain ECM include fibrous glycoproteins, such as collagen (type IV), laminin, fibronectin and tenascins, as well as several proteoglycans and hyaluronic acid[36]. Therefore, biomaterials extracted and purified from decellularized ECM, such as Matrigel, are suitable options for CNS modelling[93]. Matrigel represents a common substrate for CNS bioengineering; however, its contents are variable between batches, incompletely defined and sourced from cancerous tissues with oncogenic ECM microenvironments. Nevertheless, natural biopolymers are biocompatible and promote cell–material interactions, such as adhesion, growth, differentiation and network formation; however, rapid degradation, variable mechanical properties and cytotoxic degradation products may limit their use in CNS models.

Collagen is abundant in all connective tissues of the body, including in the CNS. Collagen type IV is most common in the brain; however, collagen type I, which can be inexpensively and readily derived from rat tail extracts, has tunable cross-linking properties that can be exploited to tailor mechanical strength, porosity and other physiologically relevant factors[93]. To promote cell–material interactions, collagen is often combined with other synthetic and natural polymers, such as hyaluronan. Although hyaluronan does not favour cell adhesion, combined with collagen in hydrogels, its high porosity and viscoelastic properties enhance survival and CNS regeneration, and promote neurite outgrowth, proliferation of neural precursors and differentiation[94]. Collagen and hyaluronan can be extracted from the ECM of vertebrates,

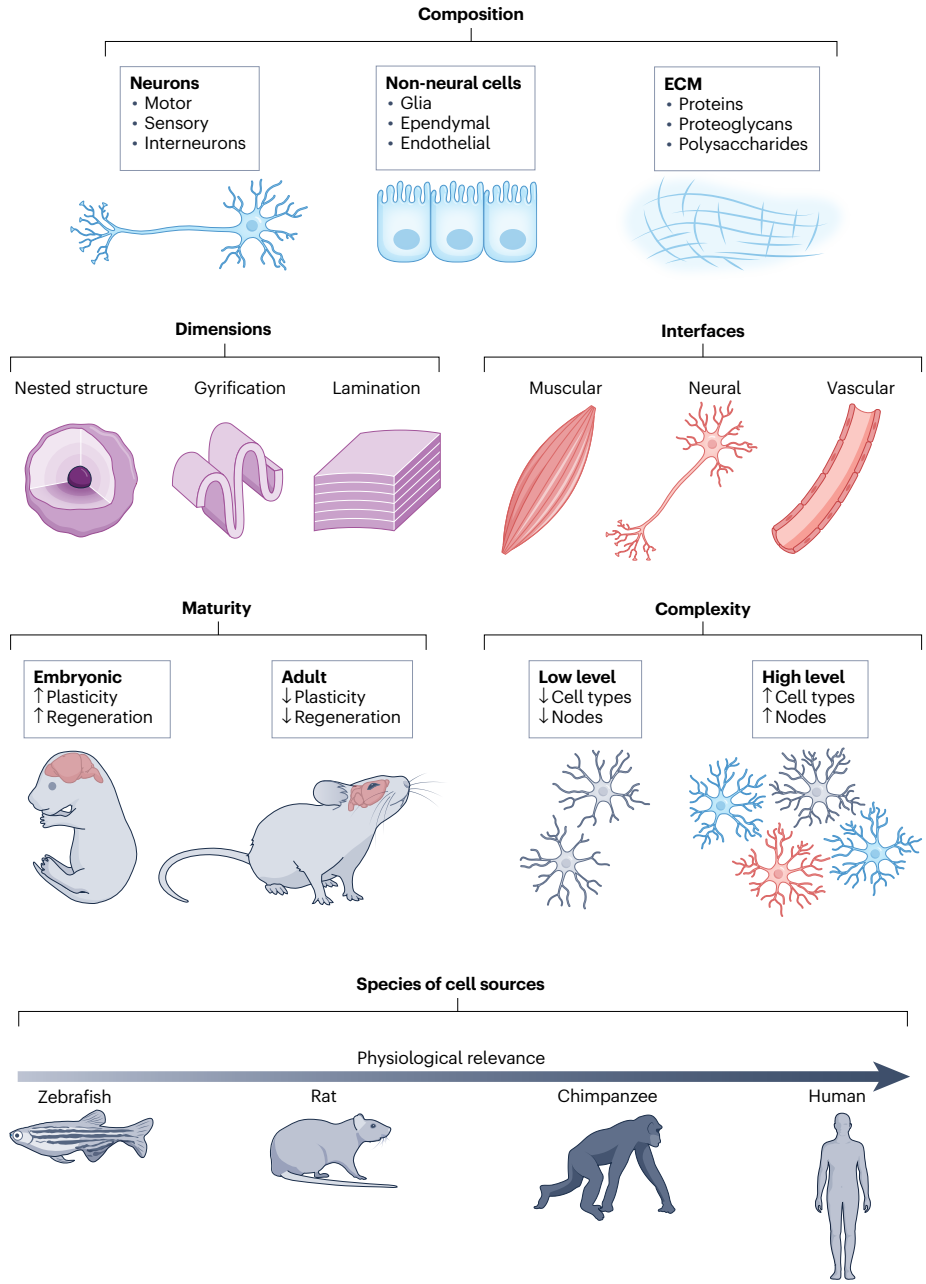

Fig. 3 | Design elements in functional central nervous system modelling. Design elements can be selected and combined to recapitulate desired physiological states in bioengineered central nervous system (CNS) tissues. The composition, dimensions, maturity, complexity and interfaces can be customized by selecting different design elements. ECM, extracellular matrix.

showing low antigenicity with minimal inflammatory response when used as grafting materials, and, similar to gelatin, agarose or alginate, represent common bioink building blocks for 3D-printed CNS models.

Chitosan is the deacetylated form of chitin, one of the most abundant natural polysaccharides. Chitin has traditionally been sourced from crustacean shells, but is also available from beetles, algae, bacteria and yeast[95]. Chitosan can be formed into sponges, gels, bioinks and scaffolds. In hydrogel form, chitosan supports cell adhesion, survival, neurite outgrowth and CNS regeneration, with anti-fungal and anti-bacterial properties[96]. Similarly, silk fibroin – derived from the cocoons of *Bombyx mori* – is a biocompatible, highly tunable biomaterial that can be formed into hydrogels with a stiffness similar to that of CNS tissues (<100 kPa)[50] and a controllable rate of biodegradation. Porous scaffolds composed of silk fibroin microfibres can be combined with collagen type I to support CNS models with synchronous network activity and compartmentalization of cell bodies and neurites[25], as well as long-term in vitro structure–function stability to study

chronic neurodegenerative disease[97]. Silk can also be combined with electrically conductive materials to integrate microelectronics[98].

Synthetic materials. Synthetic materials address many limitations of natural biomaterials, offering high mechanical stability and control over scaffold topology, fibre alignment and biodegradability[53]. Although typically less biocompatible, synthetic materials can be coated with biomaterials or infused with neurotrophic factors to enhance cell–scaffold interactions. In addition to glass, ceramics and metals, synthetic polymers such as poly(ethylene glycol) (PEG), polycaprolactone (PCL), poly-L-lactic acid (PLLA) and PEDOT represent non-biologically derived options for neural tissue engineering[99]. Of note, the hydrophilicity of synthetic biopolymers can be tuned[100] to avoid monocyte adhesion to hydrophobic material surfaces, and subsequent immune rejection of grafts.

PEG is non-immunogenic and offers tunable mechanical properties that mimic soft tissues, such as the brain[101]. For example, PEGDA hydrogels promote neurite extension to regenerate CNS tissues[102]. PCL is an elastic, slow-degrading synthetic polymer that is typically generated by electrospinning[103]. Combined with natural polymers, it is biocompatible and promotes cell adhesion; however, PCL can have cytotoxic effects if combined with organic solvents, limiting some applications, including experiments involving the dilution of water-insoluble drugs, which are common in neuropsychiatric research[104]. PLLA scaffolds made of nanoscale and microscale fibres mimic the ECM, displaying a high surface-to-volume ratio as well as high porosity and variable pore sizes, promoting cell migration, differentiation and neurite outgrowth[105]. PLLA can be biofunctionalized with natural biomaterials, which form covalent bonds with the ester linkages along its polymer backbone.

Synthetic polymers can not only mimic the neural microenvironment but also provide additional functions, such as electroconductivity. For example, PEDOT can be used to electrically record and stimulate bioengineered neural tissues, promoting differentiation of stem cells and guiding axonal outgrowth by galvanotaxis[93]. Combining PEDOT with polystyrene sulfonate (PSS) or biomaterials, such as chitosan and silk, allows the creation of biofunctionalized, electroconductive 3D printable hydrogels and scaffolds for multiple applications, including

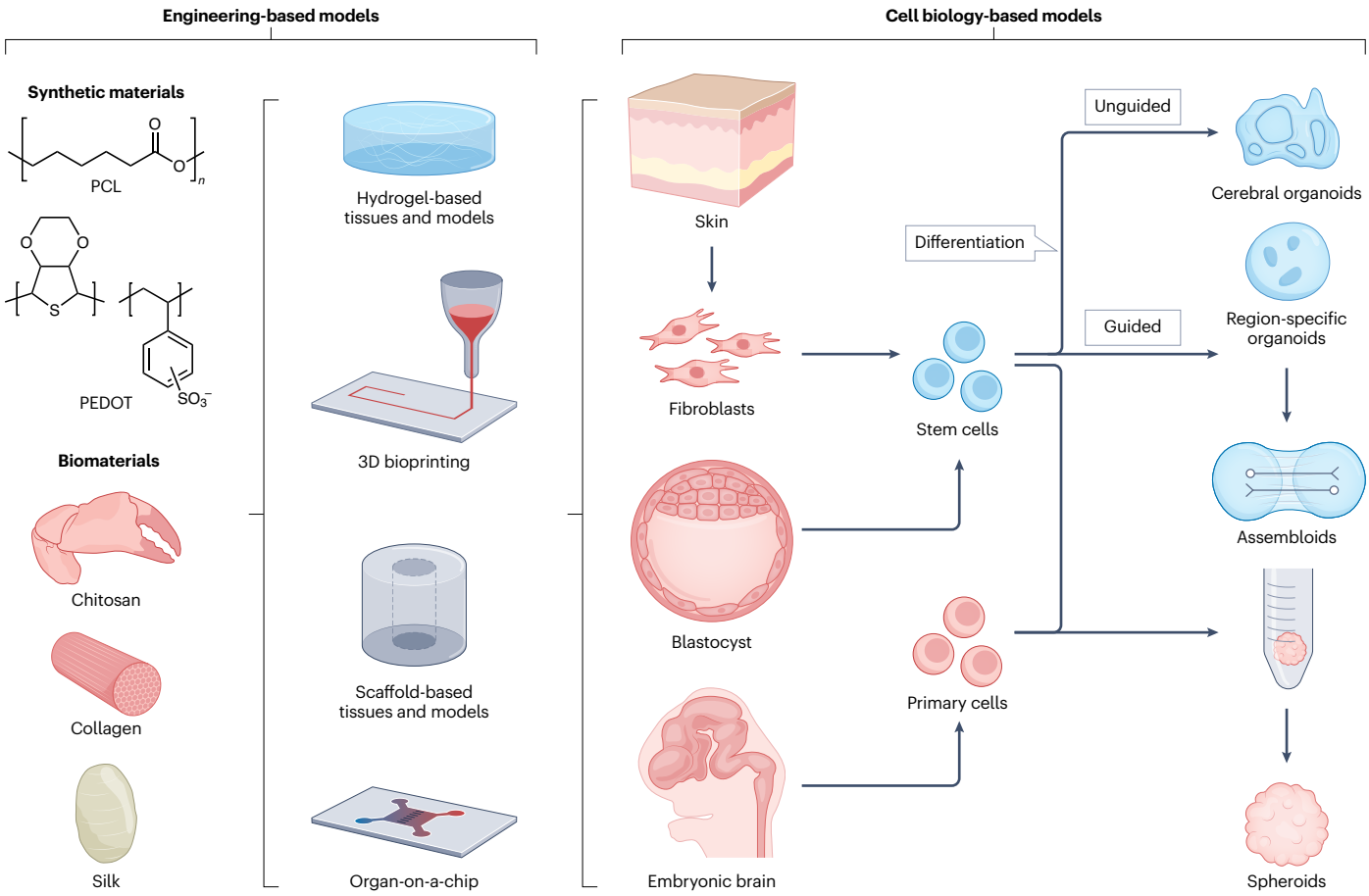

Fig. 4 | Functional 3D bioengineered central nervous system models. Engineering-based models combine materials and cell sources to generate biomimetic central nervous system (CNS) tissues. Synthetic materials (for example, polycaprolactone (PCL), poly(3,4-ethylenedixoythiophene) (PEDOT)), biomaterials (such as chitosan, collagen, silk) or combinations thereof are used to generate highly customizable and tractable hydrogels, 3D bioprintable inks, scaffolds and organ-on-a-chip systems. However, engineered models require judicious selection and micromanagement of design elements to avoid generating physiologically aberrant tissues. Cell biology-based models offer greater cytoarchitectonic complexity and ideal 3D microenvironments at the cost of some customizability. Self-assembling organoids, assembloids and spheroids, derived from pluripotent stem cells (PSCs) and primary tissues, do not require support materials, and display conserved neurodevelopmental programmes consistent with in vivo tissues.

integrated MEAs for real-time electrical monitoring[106]. Carbon nanotubes, which can also be integrated with 3D printing, have similar electrical properties to PEDOT, guiding neurite outgrowth in brain and spinal cord regeneration[93].

PDMS is a low-cost silicon elastomer amenable to high-throughput manufacturing. PDMS can serve as a mould to shape bioprinted and neurospheroidal assemblies, enabling fine control of features. In addition, PDMS is commonly used as a substrate for microfluidic CNS model systems[83]. However, the hydrophobic properties of unmodified PDMS can be problematic for tissue engineering owing to issues of cell adhesion, which can be addressed by collagen coating or topographical patterning[107].

Region-specific CNS models

Whole-brain CNS modelling was initially achieved in 2013 with an organoid system[21], and since then innovations in 3D microenvironment biomimicry have allowed the engineering of increasingly vascularized[108] and functionally competent[23,109] CNS tissues. However, to isolate and interrogate the diverse functions of neural tissues, region-specific model systems are being explored, circuits and pathways reconstructed, multiple techniques synergistically integrated and multiplexed functional read-outs developed (Table 1).

Cerebrum

Cerebral cortex. The cerebral cortex is a thin (1–4 mm), multilayered tissue (3–6 layers) that envelops the superficial folds of the cerebrum. It is divided into at least 50 subregions with distinct functional correlates, from vision and hearing to moral judgement[110]. Many functional models have been explored for the cerebral cortex, in part because it is the primary neural correlate of conscious experience[111] but also because it is particularly vulnerable to degeneration from ageing and disease. However, to increase physiological relevance and enable clinical translation, the most essential elements of cortical tissue structure–function relationships need to be captured in bioengineered models.

Cortical organoid models have long suffered from persistent embryonic-like phenotypes and poor vascularization[112]. Postnatal phenotypes can be achieved after approximately 9 months in culture, closely mirroring in vivo development and confirming the conservation of intrinsic neurodevelopmental programs in vitro[113]. Similar periods of maturation were observed in silk scaffold-based cortical models based on neurons and glia derived from iPS cells[114], which, similar to organoids, can be cultured continuously for 2 years or longer[97]. Mature, long-term cultures are key to investigating mechanisms of neurodegenerative diseases and other protracted or chronic illnesses.

Microfluidic devices provide platforms for neurovascular interfacing[18] and enable vascularization in cortical organoids. Alternatively, vascularization can be achieved through co-culture with human umbilical vein endothelial cells[115], or by transplanting organoids into immunodeficient rodents[112]. Transplanted into the brains of animals, cortical organoids[116] and scaffold-based constructs[117] form graft-to-host projections and integrate in native tissues. For example, an organoid model overexpressing the early endothelial progenitor marker ETV2 transcription factor can form perfused vascular-like structures with tight junctions, characteristic of the BBB[118]. Owing to their tunable porosity, hydrogel-based and scaffold-based cortical models can balance important 3D microenvironmental features such as the perfusion of oxygen and nutrients to facilitate nervous system repair[119]. Furthermore, neurovascular interfacing can be achieved using cortico-vascular spheroid hybrids[120] or 3D-bioprinted neurovascular tissues with laminated cytoarchitecture[58] and tunable BBB-relevant cell subtypes such as pericytes and vascular smooth muscle cells[26].

The functional competence of cerebral cortex models can be assessed by analysing electrical dynamics. For example, electric oscillations in cortical organoids driven by mature glutamatergic and GABAergic (GABA (γ-aminobutyric acid)-producing) neurons resemble human electroencephalography in preterm neonates[23]. These cortical organoids contain high glial cell concentrations and show an age-dependent decrease in progenitors. Electric responses consistent with learning can also be evoked by patterned stimulation of a silk fibroin scaffold-based cortical model embedded in a collagen hydrogel[121]. However, the functional potential of bioengineered CNS models is often underestimated and mainly based on electrophysiological techniques that were initially designed to assess 2D cultures. With the emergence of 3D MEAs[14] and other highly integrated monitoring platforms, it is now possible to assess electrical, optical, chemical and thermal parameters simultaneously. These techniques are being applied to assess the functional correlates of assembloids, which can recapitulate features of the human brain connectome including cortico-thalamic circuits[54]. Indeed, CNS models may soon be sufficiently complex to express higher-order functions, including cognition in vitro[122]. For example, the term 'consciousnessoid' implies that neural correlates of consciousness may be detectable in vitro[123].

Cortical modelling has long been limited by the absence of gyrification — the process by which the cerebrum acquires its folded morphology. To address this limitation, 4D bioprinting can be applied to combine cells with 'smart' materials that can be activated by near-infrared light or other triggers to recreate temporal changes in the cerebral structure consistent with neurodevelopment in vivo[124]. Similarly, microfluidic chips can be integrated with neurospheroids and organoids to assess the impact of cerebral fluid flow on 3D cortical folding dynamics in vitro[125]. Cortical folding in developing organisms is regulated by the stiffness of the overlaying skull and other mechanical factors[126], and therefore gyrification may be tuned by constraining tissue expansion with materials.

Hippocampus. The hippocampal bodies, which comprise interlocking c-shaped tissue structures, are located deep within the temporal lobes. Their cells receive direct inputs from the neighbouring entorhinal cortex, where pace-making stellate cells deliver a steady input of theta (~7 Hz) oscillations that couple with and phase-modulate gamma (~40 Hz) oscillations. Together, these signal patterns are crucial for memory encoding and retrieval[127]. Hippocampal bodies degenerate in patients with Alzheimer disease and other dementias, leading to impaired theta–gamma coupling and memory deficits[128]. Bilateral resection of hippocampal bodies results in dense anterograde amnesia[129].

Few models of the hippocampus have been bioengineered thus far, despite its importance as a major correlate of dementias and neurodegenerative pathologies, such as Alzheimer disease. Biomaterial-based hydrogels[130–132], bioprinted constructs[133], multichannel scaffolds[134] and microfluidic devices[135] seeded with primary hippocampal cultures allow the investigation of axon guidance, ECM-dependent viability and pathologies such as traumatic brain injury. Nanofabricated graphene oxide-functionalized scaffolds promote hippocampal cell differentiation[136], and microfluidic devices enable the creation of cortico-hippocampal circuits-on-a-chip[137].

Table 1 | Bioengineered models of CNS pathology

Pathology	Brain regions	Technique	Cell sources	Materials	Major achievement	Ref.
Neurodegenerative disorders						
Alzheimer disease	Whole cerebrum	Organoids	iPS cells derived from human peripheral blood mononuclear cells	Matrigel	High-throughput drug screening platform for Alzheimer disease using 1,300 organoids from 11 human donors, including CRISPR–Cas9-edited isogenic lines	221
	Cerebral cortex	Scaffolds, hydrogels	iNSCs derived from human foreskin fibroblasts	Silk fibroin, collagen I	Alzheimer disease phenotype induced by herpes simplex type I (HSV-1) with amyloid plaques, gliosis, inflammation and abnormal electrophysiological responses	222
Amyotrophic lateral sclerosis (ALS)	Spinal cord	Microfluidic chips	iPS cells derived from fibroblasts from patients with sporadic ALS	PDMS, collagen I, Matrigel	A contractile, neuromuscular interface-on-a-chip with cells derived from patients with ALS	179
	Spinal cord	Organoids	iPS cells, ES cells from human sources; familial ALS lines; sporadic ALS line	Matrigel	Complex, sensorimotor organoids with lower motor neuron–muscular interfaces that are impaired in tissues with ALS-linked cells	223
Huntington disease	Cortex, striatum	Microfluidic chips	Primary murine cells from wild-type, $Hdh^{CAG140/+}$ and $Hdh^{Q111/+}$ E15.5 embryos	PDMS, laminin	A novel cortico-striatal circuit-on-a-chip with defined pre-synaptic and post-synaptic compartments and Huntington disease-like phenotypes	144
	Striatum	Organoids	iPS cells derived from healthy humans and fibroblasts from patients with Huntington disease	Matrigel	Identification of overacculumated HSF1 in striatal organoid mitochondria that causes Huntington disease-like behaviours in rodents	224
Parkinson disease	Midbrain, cortex	Bioprinting, spheroids	ES cells from human and murine sources	Self-assembling peptides	Bioprinted scaffold-based model with self-assembling peptides and dopaminergic neurons from human and murine ES cells with neurotoxic lesions mimetic of Parkinson disease	225
	Midbrain	Organoids	iPS cells from patients with Parkinson disease carrying LRRK2(G2019S) mutation	Matrigel	Isolation of a neurodevelopmental defect in midbrain dopaminergic cells expressing LRRK2(G2019S) with a novel midbrain-like organoid model	226
Neurodevelopmental disorders						
Autism spectrum disorder (ASD)	Forebrain	Organoid	iPS cells derived from patients with CNTNAP2-associated ASD	Matrigel	Organoids derived from patients with ASD display progenitor-driven cortical overgrowth, which is reversible by gene editing with CRISPR–Cas9	227
Schizophrenia	Cerebral cortex	Spheroids	iPS cells derived from patients with schizophrenia and healthy controls	n.a.	Voltage-gated potassium channels (Kv4.2) identified as a contributor to decreased neuronal activity in schizophrenia using 3D neurospheroids	192
	Whole cerebrum	Organoid	iPS cells derived from human fibroblasts	Matrigel	Developmental disruption model of schizophrenia driven by abnormal WNT signalling, using cells with *DISC1* mutations	228
	Hippocampus (CA3)	Microfluidic chips	iPS cells derived from patients with schizophrenia and healthy controls	PDMS, Matrigel, laminin	Hippocampal CA3 region-on-a-chip device with reduced activity in schizophrenia-derived constructs relative to healthy controls	229
Epilepsy-related disorders						
Rett syndrome	Whole cerebrum	Organoids	iPS cells derived from patients with Rett syndrome or healthy controls	Matrigel	Organoid model of Rett syndrome with miRNA dysregulation, affecting neurogenesis, neuronal migration	230
Tuberous sclerosis	Cerebral cortex	Spheroids	iPS cells derived from patients with tuberous sclerosis or healthy controls	n.a.	Gene-edited human cortical spheroid models with *TSC1* and *TSC2* mutations exhibit epileptogenic phenotypes	231

Table 1 (continued) | Bioengineered models of CNS pathology

Pathology	Brain regions	Technique	Cell sources	Materials	Major achievement	Ref.
Stroke						
Ischaemic	Cerebral cortex	Spheroids	Primary cortical cells isolated from perinatal rats (day 1 or 2)	Agarose	Distinct stroke-like responses in eurospheroids exposed to hypoxic chambers, dependent upon cell–microenvironment binding interactions	232
	Forebrain, choroid plexus	Organoids, animal model	ES cells derived from human donors	Matrigel	Transplanted organoids promote repair and endogenous neurogenesis in a middle cerebral artery occlusion rodent model	233
Haemorrhagic	n.a.	Microfluidic chip	Immortalized human umbilical endothelial cell lines	PDMS, fibrin gel	Real-time monitoring of an arteriovenous malformation-on-a-chip model demonstrating KRAS4A-dependent leaky vascular beds, recapitulating disease hallmarks	234
Traumatic injuries						
Concussion	Cerebral cortex	Scaffold, hydrogel	Primary cortical cells isolated from embryonic mice (E16)	Silk fibroin, collagen I, laminin	Markers of neurodegeneration and inflammation identified in a scaffold-based cortical model of controlled impact	235
Diffuse axonal injury	Cerebral cortex	Microfluidic chip	Primary cortical cells isolated from perinatal rats (day 0)	Silicone	Diffuse axonal injuries-on-a-chip with high degrees of control over strain parameters and shear stress	236
Laceration	Spinal cord	Scaffold, organotypic slices	Primary spinal cord tissues from postnatal pups (day 0–5)	PLA, laminin	Nanofabricated, implantable mesh scaffold for spinal cord injury repair, assessed in vitro with organotypic slice culture	237
Cancer						
Glioma	n.a.	Bioprinting, hydrogel, spheroid	Human glioma cell lines and stem cells	Alginate, gelatin	3D-printed, glioma cell-laden scaffolds recapitulate the tumour microenvironment, promoting angiogenic transdifferentiation to endothelial cells	238
Infection-related disorders						
Neurotropic SARS-CoV-2	Choroid plexus	Organoids	iPS cells derived from human fibroblasts	Matrigel	Identification of SARS-CoV-2-sensitive cells within the choroid plexus that display marked inflammatory responses and transcriptional dysregulations	139
	Neurovascular interface	Organoids	iPS cells derived from human fibroblasts	Matrigel	Cortical–neurovascular assembloids interfaced with pericytes are preferentially infected with SARS-CoV-2 compared with cortical organoids	189

CA, cornu ammonis (sub-region of the hippocampus); Cas9, CRISPR-associated protein 9; CNTNAP2, contactin-associated protein-like 2; CRISPR, clustered regularly interspaced short palindromic repeats; *DISC1*, disrupted in schizophrenia 1 gene; E(number), embryonic gestational day; ES cell, embryonic stem cell; *Hdh*, mouse Huntington disease gene homologue; HSF1, heat shock transcription factor 1; iNSC, induced neural stem cell; iPS cell, induced pluripotent stem cell; KRAS, Kirsten rat sarcoma; Kv, voltage-dependent potassium channel; LRRK2, leucine-rich repeat kinase 2; miRNA, microRNA; n.a., not applicable; PDMS, polydimethylsiloxane; PLA, poly-L-lactic acid; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; *TSC1* and *TSC2*, tuberous sclerosis genes; WNT, Wingless, Int-1 signals.

Hippocampal organoids were first generated by adapting cerebral organoid protocols[138]. These self-organized tissues contain pyramidal neurons and granule cell types that define the cornu ammonis (CA) fields and dentate gyrus, which are essential contributors to the hippocampal circuit — a complex structure which has yet to be fully recapitulated in vitro owing to inadequate controls over cell composition and tissue polarization. Interestingly, hippocampal organoids also display interfacial features characteristic of the choroid plexuses, which generate cerebral spinal fluid within the cerebral ventricles. Such hippocampal organoids have been used to assess the neuroinvasive potential of SARS-CoV-2, as a model of CNS-related COVID-19 infection[139]. In addition, iPS cell-derived hippocampal spheroids can serve as tools for patient-tailored assessment and treatment of Alzheimer disease[140].

Basal ganglia. The basal ganglia include the putamen, caudate nuclei, nucleus accumbens and other regions that are functionally tied to involuntary movement, decision-making, learning and addiction[141]. Dysfunctions of neurons that transmit dopamine, glutamate and GABA within and between the basal nuclei underlie the signs and symptoms of Parkinson disease and other disorders[142].

The putamen and caudate nucleus, which together form the striatum, represent the most commonly modelled regions of the basal ganglia. Human striatal organoids express medium spiny neurons with GABAergic markers and postnatal electrical phenotypes[143]. They also contain glutamatergic neurons, astrocytes and oligodendrocytes. Fused with cortical organoids, these striatal organoids form cortico-striatal assembloids with functional neural circuits which become aberrant if cell sources from patients with neurodevelopmental disorders are used.

The cortico-striatal circuit can also be recapitulated in microfluidic devices, as a minimal model of striatal atrophy and network hypersynchronization in Huntington disease[144]. Similarly, a human nigro-striatal pathway-on-a-chip can be established on a compartmentalized

microfluidic platform[145]. In addition, models of the cortico-striatal and nigro-striatal pathways can be bioprinted using bioinks infused with cells that are reprogrammed to express specific phenotypes in response to cocktails of transcription factors[133].

Thalamus

CNS modelling trends towards the development of systems and pathways, and therefore models of the thalamus may enable more complex bioengineering owing to its role as a relay point, gating centre and modulator of sensory information. Thalamic neurons can be generated from mouse ES cells exposed to bone morphogenetic protein 7 (BMP7), yielding phenotypically mature cells that can innervate organotypic cultures and brains in vivo, when implanted subcortically[146]. Thalamic organoids can then be generated from human ES cells fused with cortical organoids to recapitulate cortico-thalamic circuits[147]. Remarkably, retinal organoids coupled to cortico-thalamic organoids can model visual pathway development[148]. Although bioprinted and scaffold-based models of the thalamus have not yet been reported, brain-on-a-chip models have been developed that contain cortical and thalamic co-cultures[149]. In such microfluidic devices with integrated MEAs, the activity of cortical ensembles can be monitored, which is differentially modulated by thalamic and hippocampal inputs[150].

Hypothalamus

Hypothalamic nuclei regulate autonomic functions, such as satiety, sexual behaviour, aggression, metabolism and circadian rhythms. They also interface with vasculature, endocrine organs and the cerebral ventricles to detect changes in temperature, soluble chemicals and pressure, among other signals. Mouse ES cell-derived neuroectodermal cells can be driven towards neural progenitors with hypothalamic phenotypes by removal of factors that promote growth and tissue patterning[151]. Similarly, hypothalamic neurons with ventricular-like interfacial features can be derived from human ES and iPS cells; these neurons express oxytocin, vasopressin, corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone, among others[152]. Directed differentiation protocols are also available to generate specific neuropeptidergic cell subtypes[153] and nucleus-specific hypothalamic organoids[154–156].

Organoids expressing molecular markers characteristic of the arcuate nucleus can be generated with iPS cells from healthy donors and patients with Prader–Willi syndrome[154], displaying transcriptomic dysfunctions consistent with the disorder in vivo. In addition, 3D pituitary models[157,158] may pave the way for neuroendocrine hybrids[159]. Human iPS cells can also be differentiated into functional hypothalamic–pituitary units that respond to hypoglycaemic conditions by secreting adrenocorticotropic hormone (ACTH), thus recapitulating the CRH–ACTH stress pathway[160]. Alternatively, brain-on-a-chip neuroendocrine systems are being explored[161].

Brainstem

CNS models of the medulla, pons and the midbrain remain limited; however, owing to the clinical relevance of the substantia nigra and the ventral tegmental area as key anatomical correlates of neuropsychiatric disorders, such as Parkinson disease and schizophrenia, brainstem organoids have been developed[162,163]. These midbrain-like organoids express neuromelanin pigmentation and dopaminergic profiles consistent with substantia nigra structure and function. Midbrain organoids can recapitulate pathophysiological characteristics of Parkinson disease, including network dysfunction and α-synuclein aggregation[164,165].

Heterogeneous brainstem organoids can be created from human iPS cells with a strong midbrain-like phenotype and dopaminergic cells[166]. Brainstem organoids express specific cholinergic (choline acetyltransferase (ChaT)) and noradrenergic markers (dopamine β-hydroxylase) as well as hindbrain-specific genes (zinc finger of the cerebellum 1 (ZIC1), ZIC4), and therefore likely contain pontine and medullary cells. Moreover, hindbrain organoids can be derived from human iPS cells and serotonergic cells with genetic profiles congruent with genes expressed in the raphe nuclei of the medulla[167]. Engineering-based models of the brainstem have not yet been developed.

Cerebellum

The cerebellum, similar to the cerebrum, comprises several embedded nuclei surrounded by dense tract systems and a thin, three-layered outer shell of cortical tissue with high gyrification; however, its unique microcircuitry greatly differs from that of the cerebral cortex. Engineering-based models of the cerebellum have not yet been developed, but bioprinting strategies, explored for the generation of laminar morphology and gyrification in the cerebral cortex, may also be adapted for cerebellar modelling.

Cerebellar organoids with self-organized and polarized 3D microanatomy can be generated using human ES cells with populations of functional Purkinje cells; however, their morphologies remain phenotypically embryonic with neural tube-like features[168]. Alternatively, spinning bioreactors can be integrated to generate cerebellar organoids with mature cytoarchitectures[169]. In addition, spheroids and organoids can be created by exposing human iPS cells to morphogens, including retinoic acid, Wingless, Int-1 signals (WNT) and sonic hedgehog (SHH), which promote cerebellar differentiation. These cell biology-based cerebellum models express markers of multiple cortical layers and show normal electrophysiological signatures of Purkinje cell function[170].

Spinal cord

Modelling the spinal cord is particularly interesting for the development of regenerative therapies for spinal cord dissection, degeneration, atrophy and other pathologies. The spinal cord has a rather simple internal cytoarchitecture and circuitry, and thus both cell biology-based and engineering-based models of the spinal cord could be developed.

Spinal cord tissues can be 3D-printed using a fibrin-based bioink infused with differentiated neural progenitor cells derived from human iPS cells, ultimately expressing lower motor neuron markers indicative of neural cell fates consistent with the ventral spinal cord[171]. Similarly, chitosan, hyaluronan and Matrigel can provide fast-gelling bioinks to construct implantable, NSC-laden, 3D-printed scaffolds to promote axonal regeneration and decrease scarring after an experimental spinal cord injury in living rodent models[172]. A scaffold–organoid hybrid method can be bioprinted using a gelatin-based, enzymatically crosslinked hydrogel bioink infused with boundary cap neural crest stem cells to construct 3D spinal cord tissue[173]. Importantly, bioprinting allows the fabrication of cell type-specific tissues[174] with single-cell precision and the infusion of printed scaffolds with growth factors and signalling molecules[58], thus potentially enabling the construction of 3D spinal circuits that underlie the basic reflexes involving compartmentalized motor neurons, interneurons and sensory neurons.

Lower motor neuron-containing neuromuscular junction circuits can be designed on microfluidic devices[175,176], for example, using

optogenetically excitable motor neurons differentiated from mouse ES cells within a 3D collagen–Matrigel hydrogel. Such systems allow the study of degenerative disorders, such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy[177]. In particular, compartmentalized 3D ALS-on-a-chip models are promising tools for clinical research[178,179]. More complex, highly parallelized spinal circuits can also be generated in microfluidic devices by guiding the axons of multiple motor neuron spheroids towards myofibre targets[180].

In addition, cerebral organoid protocols can be adapted to generate spinal cord organoids[181] with motor neurons, interneurons, spinal astrocytes and embedded morphogen gradients, consistent with the rostro-caudal axes of the ventral horns of spinal cords in vivo. Interestingly, by modulating concentrations of BMP4 and SHH, dorsal and ventral-intermediate spinal cord organoids can be generated with distinct sensory and motor neuron populations, respectively[182]. Thus, sensory-motor circuitry could be realized by fusing dorsal and ventral segments[183]. Such modular tissues can be combined to form complex, neuromuscular assembloids to study neural tube defects[184].

Retinal tissues

The retina is a multilayered neural tissue situated deep within the eye. The retina contains various neuronal subtypes, photoreceptive cells and ganglion cells, whose axons form the optic nerves that initiate signalling within the visual pathway. The first cell biology-based model of the retina was generated by aggregation of dissociated cells from mouse and chick tissues into internally laminated retinospheroids[185]. Additional tissue patterning, including pigmentation and centre-surround polarization, was later achieved in retinal organoids derived from ES cells by stepwise differentiation protocols with transient morphogen exposures[186]. Retinal organoids can also be further functionalized with photoreceptors that actively respond to light.

The migratory behaviour of human and non-human retinal lineage cells can be explored in microfluidic devices that contain chemical gradients, tissue geometry and ECM substrates[187,188]. Moreover, bioprinted hydrogels based on hyaluronan can recapitulate biophysical features of the retinal microenvironment, promoting differentiation of photoreceptors from retinal progenitors[189]. Similarly, photoreceptors can be precisely printed as layers on top of retinal pigment epithelium[190]. To create tissue-guiding moulds for retinal research, synthetic scaffolds can be 3D-printed with hexagonal arrays of pores using two-photon polymerization; the scaffolds can then be seeded with retinal progenitor cells, which differentiate into retinal neurons[191].

Olfactory tissues

Olfactory epithelia, bulbs and nerves transduce chemical energy and transmit signals to distinct nuclei and cortical regions, where smell is ultimately perceived. Although implicated in several psychiatric and neurological disorders, the olfactory epithelium has received little modelling attention thus far, compared with other CNS tissues. Olfactory neurospheroids can serve as models of schizophrenia[192], autism spectrum disorder (ASD)[193] and Parkinson disease[194]. In addition, bioprinted scaffold-based models[195] and organoid models of the olfactory epithelium are being explored[196,197].

Neural-X interfaces

Muscular. Neuromuscular interfaces underlie all behaviours, including speech, facial expression and basic spinal reflexes. Unlike in living organisms, disembodied 2D or 3D neural tissues are unable to physically interact with their environments. Therefore, investigations of behavioural phenomena characteristic of neurological disorders have historically been limited to the study of animals. Alternatively, neuromuscular assembloids can provide in vitro platforms for the investigation of corticospinal function, featuring definable upper (cortical) and lower (spinal) motor organoids as well as excitable musculature[55]. In these 3D assembloids of the pyramidal tracts, key features of diseases of the neuromuscular junction, such as myasthenia gravis, can be modelled[198]. Similarly, cortico-striatal models can recapitulate the extrapyramidal system to study ASD, obsessive-compulsive disorder and Parkinson disease[141].

A neuromuscular interface-on-a-chip can be engineered using a microfluidic device and spheroids made from cells from patients with ALS to model axonal regression, motor neuron death and muscle atrophy in vitro[179]. Similarly, a developmental model of the neuromuscular junction can be generated by combining human skeletal muscle cells and ES cell-derived motor neuron clusters in a hydrogel, followed by seeding in PDMS moulds, which results in self-organized 3D tissues that express cholinergic neurotransmission[199]. Such engineering-based models[200] may serve as high-throughput tools for drug screening and personalized medicine to combat neuromuscular disease.

Sensory. Perceptual modalities, such as vision, hearing and somatosensation, allow organisms to avoid threats and pursue positive fitness outcomes. These modalities rely on the transmission of sensory information from the peripheral nervous system to the CNS. However, the role of sensory input as a determinant of cultured neural network function is often overlooked. Disembodied neurons display physiologically aberrant electrical activity in vitro[201], which can be rescued by applying patterned stimulations[201,202].

Several 3D neural–sensory interfaces have been developed. For example, forebrain organoids can be engineered by assembling optic vesicles as primordial eye fields with lens-like cells, corneal tissue, retinal progenitor cells and pigment epithelia[203]. These optic vesicle-containing brain organoids are functional and sensitive to different intensities of photostimulation. In addition, organoid models of the inner ear have been developed that contain functional hair cells[204,205]. A CNS–peripheral nervous system model, generated with specialized dorsal horn sensory neurons responsive to μ-opioid receptor activators and inhibitors, can be applied to study ascending spinal sensory pathways[206]. Similarly, neuro-mesodermal assembloids and 3D-bioprinted somatosensory constructs respond to capsaicin and menthol, recapitulating the receptive features of several pathways governing pain, temperature and taste[207,208].

Robotic. Neural models can also be functionally coupled to machines. For example, neural networks cultured on MEAs can serve as neural–machine interfaces to control mobile robots[209], mechanical limbs[201,210], virtual aircrafts in flight simulators[211] and virtual animal avatars[210]. Robots and other non-neural effectors can be equipped with dynamic sensors to provide patterned sensory feedback on cells and tissues contingent upon action[212]. The resulting sensory-motor feedback loops may underlie basic mechanisms of cognition[213,214]. These hybrid robots, or 'hybrots', can be based on cell monolayers or 3D neural tissues. For example, thick (0.5 mm) organotypic slice cultures can be coupled to robots using MEAs[215]. 3D CNS models and organotypic slice cultures are similar in scale and equally compatible with available electrical and optical sensors, and therefore hybrots may be controllable by bioengineered neural networks[122,216,217] to improve our operational understanding of learning and inspire the design of neuromorphic artificial intelligences[122].

Outlook

Bioengineered models of the CNS are powerful tools for basic and applied research. However, many engineering challenges remain to maximize the translational potential of CNS models. Despite the development of promising vascularization strategies, oxygenation and nutrient transport issues remain crucial bottlenecks in cell biology-based models. The seamless integration of functional vessels will likely require micromanagement of tissue patterning to mimic highly parallel and autonomous processes which are typically expressed during embryonic development and later suppressed as organisms mature. Therefore, there is a need to develop engineering approaches that activate intrinsic developmental programmes to achieve integrated tissue phenotypes. Until new perfusion strategies are developed, existing hybrid systems that combine 3D tissues with multi-compartment microfluidic devices and other vascular interfaces can support spheroid and organoid research towards increasingly personalized brain models to assist with the diagnosis and treatment of patients with neuropsychiatric diseases. The brain is among the most vascularized organs in the body and, therefore, vascular interfaces are key to the maturation of CNS models.

Bioprinting strategies, customizable scaffolds, complex microfluidic channels and other engineering-based techniques offer high spatial and temporal control over bioengineered CNS tissues. However, these approaches suffer from printing shear stress, a lack of appropriate bioinks, low cell densities, imprecise tissue architectures and low microenvironmental relevance. In particular, ECM composition and fine structure, including the integration of proteoglycans, perineuronal nets and biophysical cues, remain undervalued. Drawing inspiration from organization principles found in nature, such as molecular self-assembly, disorder to order processes and diffusion reactions, complex material designs could be implemented, ensuring a balance of physiological relevance and tractability for high-throughput applications[72]. However, model systems remain specialized tools, and multiple approaches are needed to overcome their intrinsic limitations. For example, the physiological relevance of organoids can be combined with the controlled perfusion of microfluidic devices into a superior, hybrid model system.

Engineered CNS tissues are often assembled by self-organization of stem cells, which co-opts endogenous embryonic-like morphogenetic programmes. However, mature neural circuits with postnatal phenotypes[218] would better recapitulate the physiology of later stages of ontogeny, including puberty and senescence, with age-matched regenerative competence, and thus substantially improve the translational potential of bioengineered CNS tissues. Indeed, drug development and repurposing, injury repair and even the reversal or suppression of normal ageing processes are dependent upon progress in this area[219].

Finally, higher degrees of functional complexity in CNS models, and coupling with sensory and motor interfaces, would allow the in vitro assessment of reflex arcs, associative learning and other rudimentary sensory-motor phenomena. Thus, CNS models may serve as platforms to test hypotheses related to embodied and minimal cognition, elucidating the mechanisms and evolutionary origins of intelligence and other higher-order brain functions. Building brain models with cells and other biological building blocks derived from diverse species may enable comparative analyses of cognitive function in vitro. Bioengineered tissues may also facilitate the design of neuromorphic computers and bioinspired artificial intelligence. Of note, CNS functions also include a capacity for sentience as well as the experiences of pain and suffering, which raises ethical and legal concerns related to embodied or minimally cognitive neural systems[123,220]. Therefore, ethical and legal frameworks should be developed for bioengineered CNS tissues; however, the full impact of higher functional competence in CNS models may not be fully appreciated until they are built and measured.

Citation diversity statement

The authors acknowledge that papers authored by scholars from minoritized groups are systematically under-cited. Here, we have made every attempt to reference relevant papers in a manner that is equitable in terms of racial, ethnic, gender and geographical representation.

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