Biomaterials Translational

Progress in spinal cord organoid research: advancing understanding of neural development, disease modelling, and regenerative medicine

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Key Words:
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From the Contents

ABSTRACT

Stem cell-derived spinal cord organoids (SCOs) have revolutionised the study of spinal cord development and disease mechanisms, offering a three-dimensional model that recapitulates the complexity of native tissue. This review synthesises recent advancements in SCO technology, highlighting their role in modelling spinal cord morphogenesis and their application in neurodegenerative disease research. We discuss the methodological breakthroughs in inducing regional specification and cellular diversity within SCOs, which have enhanced their predictive ability for drug screening and their relevance in mimicking pathological conditions such as neurodegenerative diseases and neuromuscular disorders. Despite these strides, challenges in achieving vascularisation and mature neuronal integration persist. The future of SCOs lies in addressing these limitations, potentially leading to transformative impactions in regenerative medicine and therapeutic development.

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Introduction

The construction and application of stem cell-derived organoids have become a cutting-edge and hot topic in the study of tissue and organ development patterns and pathological mechanisms. Organoids are three-dimensional (3D) stem cell populations capable of self-renewal and differentiation, with the ability to differentiate into multiple specific cell subtypes representative of organs.[1] They exhibit similar spatial configurations to their corresponding organs and recapitulate part of the functions. The development of organoids has significantly propelled the advancement of biomedicine, serving as an effective model for drug efficacy evaluation and biocompatibility testing. Moreover, they hold prospects for developmental biology, pathological studies of diseases, and particularly for the field of regenerative medicine.[2-4] In recent years, *in vitro* models have been established through organoid technology to simulate the early stages of spinal cord development and investigate pathogenic genes and potential therapeutic strategies related to spinal cord disorders.[5]

In this review, we aim to introduce the latest advancements in the field of spinal cord organoids (SCOs), and explore their critical role in modelling spinal cord development and disease mechanisms. Our objectives encompass a thorough summary of methodological progress in regional specificity and cellular diversity. We highlight the integration of technologies such as biofunctional materials, 3D printing, organ-on-a-chip, and assembloids in refining the structure and function of SCOs. We also delve into the application of SCOs as disease models, discussing their utility in simulating spinal cord development, neurodegenerative diseases, and neuromuscular disorders, their integration with host damaged tissue as grafts, as well as their predictive capabilities in drug

screening. Furthermore, we address the challenges faced by SCOs in achieving vascularisation and neuronal maturation, outlining future research directions aimed at overcoming these limitations. In summary, this review seeks to provide a holistic perspective to assess the current status and future potential of SCOs in spinal cord research and their translational applications in clinical settings.

We conducted a literature search using the key words of 'spinal cord organoid', 'spinal cord', 'neural tube', 'spinal cord like tissue', 'neural organoid' in the Web of Science and PubMed databases for articles published between 2017 and 2024. In cases where the literature covered similar topics, preference was given to publications in the most recent or more authoritative journals.

Advances in Spinal Cord Organoids Research

Over the past decade, significant advancements have been made in the field of neural organoid research. In 2013, Lancaster et al.[6] pioneered the development of a cerebral organoid (CO) culture system derived from human induced pluripotent stem cells (iPSCs), leveraging this novel model to dissect the pathogenic mechanisms underlying microcephaly. Subsequent research has been extensively dedicated to the generation of region-specific organoids, aiming to study the developmental patterns and tissue functions of distinct regions within the central nervous system (CNS). In recent years, scientists have successfully engineered region-specific organoids that mimic the retina, hippocampus, thalamus, midbrain, and cerebellum.[7-9]

However, the complex structure of spinal cord, comprising dozens of neuronal subtypes and 30 segments along the anterior-posterior (A-P) axis, poses a challenge for the generation of SCOs. The intricate 3D architecture of the spinal cord arises from the orchestrated spatiotemporal gradients of morphogens during embryonic development (**Figure 1**). These gradients establish the A-P, dorsal-ventral (D-V), and medial-lateral (M-L) axes, which are critical for the proper segmentation and neural differentiation of the spinal cord. The A-P axis is specified by the opposing gradients of retinoic acid (RA) from anterior, fibroblast growth factor and growth differentiation factor from posterior.[10] This axis is characterised by segmental overlapping expression of the *HOX* gene family[11] (**Figure 1A**). The D-V axis is patterned by the interplay of bone morphogenetic protein and Wnt signals emanating from the dorsal roof plate and the ventralising signal, sonic hedgehog (Shh), from the ventral floor plate[12] (**Figure 1B**). This axis is instrumental in the formation of diverse neuronal subpopulations, which emerge from 11 distinct progenitor domains. The M-L axis is shaped by the radial migration and differentiation of neural progenitor cells, and result in the intricate organisation of the spinal cord's gray and white matter, essential for its functional integration within the nervous system[13] (**Figure 1C**). This precise orchestration of developmental cues ensures the formation of a highly organised and functionally specialised spinal cord.

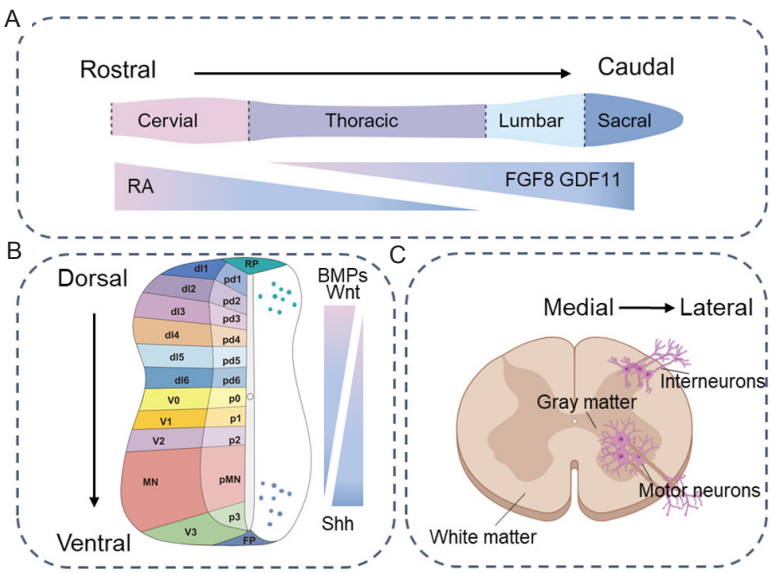

Figure 1. The developmental axes of the spinal cord are orchestrated through precise signalling gradients. (A) The A-P axis is patterned by the intersecting gradients of RA and FGF8/GDF11. (B) The D-V axis is established by the opposing gradients of BMP/Wnt and Shh. (C) The M-L axis arises from the radial migration and differentiation of neural progenitors, resulting in the formation of the gray and white matter of the spinal cord. Created with BioRender.com and Adobe Illustrator 2024. A-P: anterior-posterior; BMP: bone morphogenetic protein; D-V: dorsal-ventral; dI: dorsal interneuron; FGF: fibroblast growth factor; FP: floor plate; GDF: growth differentiation factor; M-L: medial-lateral; MN: motor neuron; pMN: motor neuron progenitor; pd: progenitor domain; RA: retinoic acid; RP: floor plate; Shh: sonic hedgehog.

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Researchers have engineered SCOs with certain tissue morphologies and functions by simulating the induction of morphogens during spinal cord development. Researchers induced tissues exhibiting characteristics of the hindbrain and spinal cord by supplementing with signals that promote caudal neural tube development.[14] In 2014, mouse embryonic stem cells were induced to form 3D neural epithelium-like tissues,[15] which could adopt gene expression patterns indicative of cervical segments with RA induction. Concurrently, studies have deciphered the developmental trajectories of the neural tube in response to specific small molecules.[16] Libby et al.[17] generated axial elongation of caudalised human organoids with regional *HOX* gene expression by Wnt agonists. These organoids, which consist of neuromesodermal and neural epithelial cell, provide a more holistic representation of the early developmental features of spinal cord tissue, offering significant insights for the development of segment-specific SCO models. In 2018, the induction of dorsal and ventral SCOs from iPSCs was achieved using bone morphogenetic protein 4 and Shh pathway agonists, respectively. These organoids gained dorsal or ventral signalling centres, differentiated into various spinal precursor subtypes, and organised into distinct progenitor domain structures.[18, 19] Additionally, researchers found that the exposure of organoids to morphogens, in terms of concentration and duration, can significantly influence the regional neuronal fate determination within these structures.[19]

Recent research has unveiled a new model of spinal cord caudalisation. This model confirms that the spinal cord tissue primarily originates from neuromesodermal progenitors (NMPs), which are located at the node-streak border and the adjacent caudal lateral epiblast.[20] NMPs can detect the co-expression of early mesodermal and neuro-precursors' biomarkers. These cells migrate to the anterior region of neural tube to develop ventral neural tissues, and then merge with the dorsal neural tissues from the anterior neural plate. NMPs are capable of producing both dorsal and ventral regions at the posterior end of the spinal cord. The proposition of this developmental model provides a new theoretical basis for the construction methods of SCOs. Whye et al.[21] have elucidated a neuromesodermal organoids generation protocol, that hinges on the induction of WNT and fibroblast growth factor signals to acquire early neuromesodermal characteristics. The method capitalises on the synergistic effects of RA signalling and Shh activation to shape the neural-tube like structure. Ultimately, the inhibition of the Notch signal expedites the acquisition of terminal cell identities. Gribaudo et al.[22] developed a method for generating SCOs that recapitulate the overall morphogenesis of the A-P axis, exhibiting multi-lineage somite development and achieving relatively mature cellular phenotypes. These organoid models serve as powerful tool for investigating multi-lineage developmental processes and for studying complex neurodevelopmental disorders, such as neural tube defects.

The spinal cord plays a pivotal role in transmitting and processing neural signals, with these critical functions relying on diverse subtypes of neurons. Single-cell sequencing has revealed that within mouse spinal cord tissue, there are 15 non-neuronal and 69 neuronal subclasses.[23] Except for neurons, other cells in CNS including oligodendrocytes, microglia, and astrocytes support normal activity of neurons, regulate the stability of the external environment, and participate in immune responses and inflammation modulation. Neuronal subtypes encompass various dorsal, intermediate, and ventral excitatory neurons, as well as inhibitory neuron clusters. Excitatory neurons in the spinal cord tissue are involved in the transmission of excitatory signals, activating target neurons or muscles. These neurons transmit neural impulses by releasing excitatory neurotransmitters such as glutamate, which bind to receptors on target neurons or muscle cells, and trigger the propagation of nerve impulses.[24-26] In contrast, inhibitory neurons suppress the transmission of neural impulses by releasing inhibitory neurotransmitters like γ-aminobutyric acid or glycine, binding to receptors on target neurons.[27-29] Excitatory and inhibitory neurons from different regions form a complex network within the spinal cord, and work together to modulate neural signals, thus achieving the precise function of signal reception and transmission in the spinal cord.

According to the developmental patterns of the spinal cord, adult spinal cord tissue contains a variety of somatotopically organised motor neurons and interneurons. Motor neurons are the neuronal population in the spinal cord responsible for transmitting neural signals to muscles. Based on their location and target muscle groups, they can be further classified as follows: α-motor neurons have axons that directly connect to muscle fibres and control muscle contraction and force regulation by releasing acetylcholine;[30, 31] β-motor neurons are connected to smaller muscle fibres and control fine muscle movements such as those of the fingers; γ-motor neurons modulate the state of muscle contraction, regulating muscle tone and stretch reflexes by releasing acetylcholine.[32-34] The dorsal and intermediate regions of the spinal cord contain interneuron subclasses that participate in spinal neural signalling, coordinating pain conduction, muscle movement, and posture regulation.[35-38] In addition to these two most common types of neurons, the spinal cord also includes sensory neurons that transmit sensory information and autonomic neurons that control the autonomic functions of visceral organs. By coordinating the interactions among various types of neurons, the spinal cord can achieve accurate signal processing and motor control.

Several cell subtypes in spinal cord mentioned above have captured the interest of researchers, including motor neurons and oligodendrocytes, which are essential for the functional integrity and study of human spinal cord developmental pattern. Seo et al.[39] utilised iPSCs to construct motor neuron organoids that exhibit properties analogous to NMPs. Exposing these organoids to hydrogen peroxide induces axonal degeneration and apoptosis in motor neurons, effectively simulating the acute oxidative stress injury observed in spinal tissue. This model serves as a valuable tool for screening pharmacological agents that promote axonal regeneration. In the context of myelin, James et al.[40] employed patient-derived cells with neurofascin-155 (*Nfasc155*)$^{-/-}$ deficiency (**Figure 2A**, and **B**), to model a monogenic disorder, and utilised spinal cord-patterned myelinating organoids, termed "myelinoids", myelin can be detected in the myelinoids (**Figure 2C–F**). iPSCs with *Nfasc155*$^{-/-}$ derived myelinoids can replicate impaired paranodal junction formation (**Figure 2G**, and **H**). This research offers a platform for investigating the development and pathological aspects of myelin.

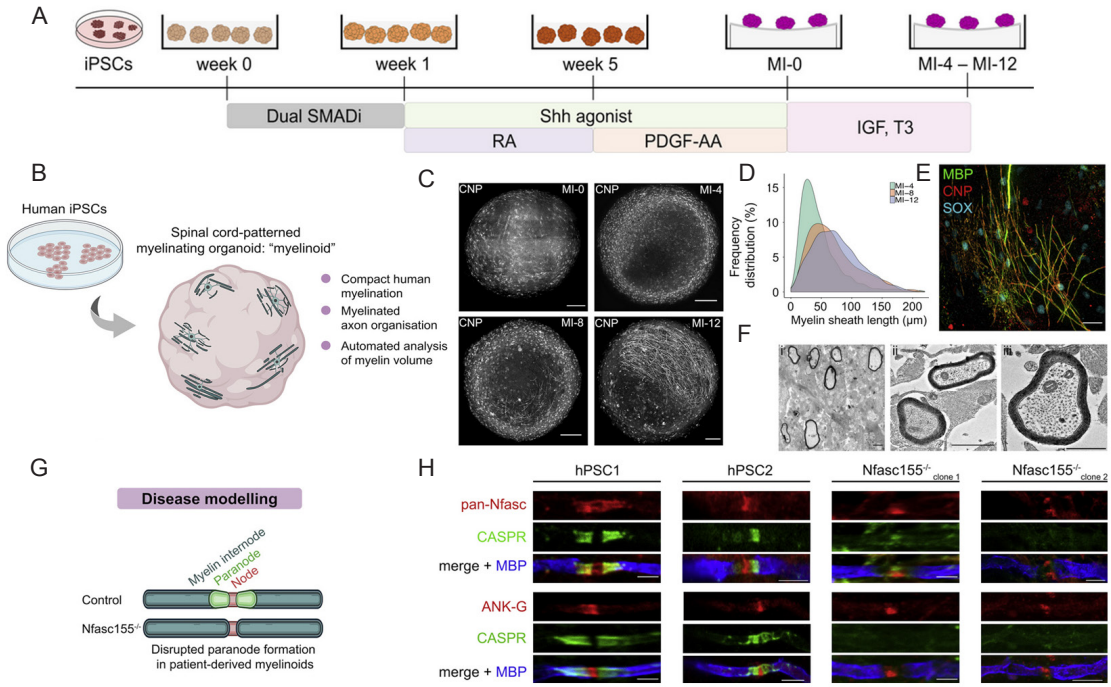

Figure 2. Generation of myelinoids and their application in disease research. (A) Schematic protocol for the generation of myelinoids. (B) Application of myelinoids in studying human myelin biology. (C, D) Distribution and quantification of myelin within myelinoids at various stages of cultivation. Scale bars: 250 µm. (E) Immunofluorescence staining analysis of oligodendrocyte morphology within MI-12 myelinoids. Scale bar: 25 µm. (F) Transmission electron microscopy images of myelinated axons within myelinoids. Scale bars: 1 µm (F1 and F2), 500 nm (F3). (G, H) Application of myelinoids in modelling disordered myelinated axon organisation caused by *Nfasc155*$^{-/-}$ mutation. Scale bars: 2 µm. Reprinted from James et al.[40] ANK-G: ankyrin-G; CASPR: contactin-associated protein-like 2; CNP: 2′,3′-cyclic nucleotide 3′ phosphodiesterase; IGF: insulin like growth factor; iPSCs: induced pluripotent stem cells; MBP: myelin basic protein; MI: myelin induction; Nfasc155: neurofascin-155; pan-Nfasc: pan-neurofascin; PDGF: platelet-derived growth factor; RA: retinoic acid; Shh: sonic hedgehog; SMADi: small mothers against decapentaplegic (Smad) inhibitors; SOX: SRY-box transcription factor; T3: triiodothyronine.

Application of Spinal Cord Organoids in Disease Modelling

Prior to the advent of SCOs technology, researchers utilised monolayer neural cells cultured on plates or animal models with surgical or genetic defects to study spinal cord-related diseases. Compared to these models, SCOs offer numerous advantages.[41] SCOs can dynamically simulate the formation of the neural tube including a diverse cellular composition of A-P and D-V characteristics, making them a powerful tool for studying spinal cord development.[17] Additionally, SCOs display a cellular diversity similar to spinal cord tissue, including neurons and glial cells, and vascular cells, which is difficult to achieve in monolayer cell cultures. Compared to animal models, SCOs as disease models can shorten the research duration and reduce the complexity of experiments. SCOs can be used to model spinal cord diseases, such as neural tube defect organoids generated through genetic editing, holding broad prospects for personalised medicine (**Figure 3**).

Spinal cord related disorders, encompassing developmental diseases, neurodegenerative disorders, spinal cord injury (SCI), and vascular pathologies, variably impair the transmission of neural signals, thereby affecting sensory and motor functions. The pathogenic mechanisms underlying these diseases remain incompletely understood, with genetic factors, environmental influences, and immune system anomalies contributing to disease progression. Currently, researchers have successfully generated SCOs that correspond to various spinal cord pathologies using organoid technology, providing insights into the underlying pathological mechanisms and holding broad prospects for personalised medicine and drug screening (**Figure 4**, and **Table 1**).[42-50]

Developmental disorder of the spinal cord

During the early stages of normal embryonic development, the neural tube gradually closes to form the spinal cord and brain. Spina bifida is a common developmental disorder of the spinal cord, where the neural tube closure process is disrupted, leading to exposure of the spinal cord and nerve tissues. This exposure can cause mechanical injury and pressure on the spinal cord and nerve roots, leading to neurological dysfunction. Additionally, patients may present with abnormalities in the tissues surrounding the neural tube, such as spinal fractures, enlargement of the spinal canal, abnormal vertebral joints, or spinal curvature.[51] SCOs provide an appropriate research platform for the development-related defects. Shin et al.[42] generated necrotic core-free human SCOs (hSCOs), which were exposed to dichlorocobalt ($CoCl_2$) to simulate the hypoxic and hypoglycaemic conditions of fetal

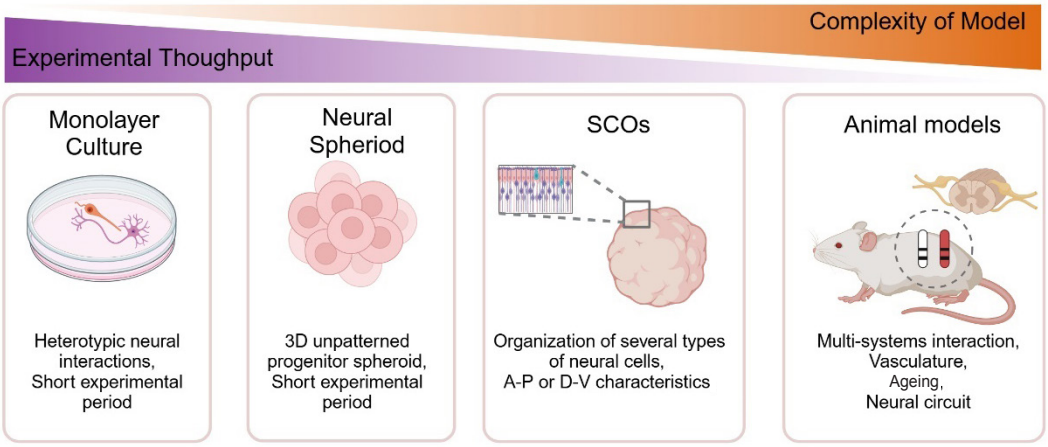

Figure 3. Different models for the research of spinal cord related disorders. Mono layer cultured neural cells can represent heterotypic cellular interactions, with short experimental period; Neural spheroids are 3D unpattern neural progenitor cells; SCOs can represent developmental characteristics of neural tube, and contains several types of neural cells; Animal models have the interaction of multi-system and vasculature, represent high complexity of physiological. Created with BioRender.com. 3D: three-dimensional; A-P: anterior-posterior; D-V: dorsal-ventral; SCOs: spinal cord organoids.

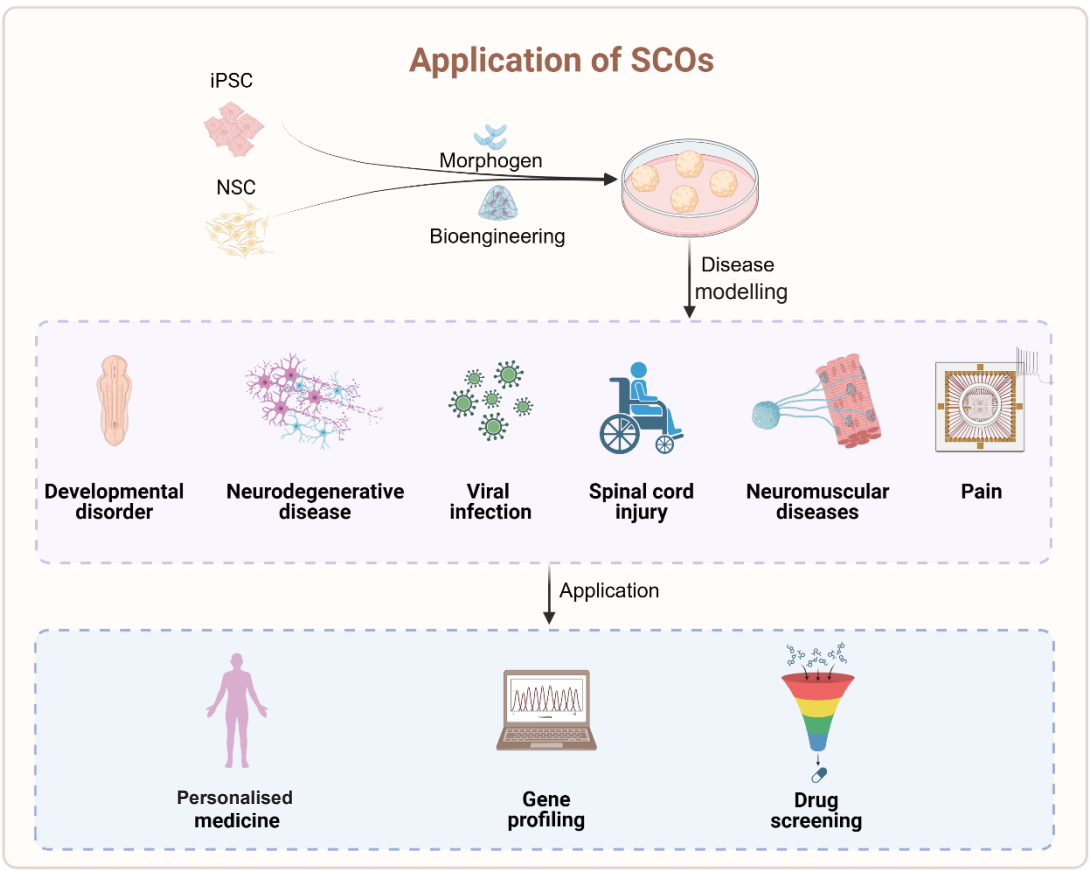

Figure 4. Construction of SCOs and their application in disease modelling. SCOs derived from iPSCs or NSCs, are cultivated using neurogenic induction molecules or bioengineering approaches. These organoids provide a robust model system for the study of a spectrum of spinal cord pathologies. Their utility extends to the elucidation of disease mechanisms and the high-throughput screening of therapeutic small molecules, underscoring their pivotal role in advancing spinal cord research. Created with BioRender.com. iPSC: induced pluripotent stem cells; NSC: neural stem cell; SCOs: spinal cord organoids.

Table 1. Applications of SCOs in disease modelling

Type of SCOs	Model establishment method	Disease modelling/phenotypes gained	Reference
nf-hSCOs	$CoCl_2$ simulation	The hypoxic and hypoglycaemic conditions of fetal spinal cord.	42
hSCOs	VPA treatment	Neurodevelopmental defects, impaired expression of tight junction proteins ZO1 and ZO2 can be detected	43
hSCOs	FUS-KO	Impaired motor neuron development	44
SCOs	VPA and carbamazepine treatment	Dose-dependent effect of antiepileptic drugs on neurotube closure defects	45
hSCOs	iPSCs from MEALAS patients	MEALAS modelling, including phenotypes of delayed motor neuron development and axonal growth defects	46
NMOs	iPSCs with *C9orf72* mutation from ALS patients	ALS modelling, including phenotypes of muscular dystrophy and loss of Schwann cells	47
hSCOs	EV-D68 infection	Viral-induced neurological disorders, including structural disruption and cellular apoptosis	48
SCOs	iPSCs from patients with SMA	Early state of SMA, including developmental bias towards mesodermal progenitors and muscle cells	49
SCO-on-chip	Modulating nociceptive molecules treatment	Pain research	50

Note: ALS: amyotrophic lateral sclerosis; C9orf72: chromosome 9 open reading frame 72; $CoCl_2$: dichlorocobalt; EV: enterovirus; FUS: fused in sarcoma; hSCOs: human SCOs; iPSCs: induced pluripotent stem cells; KO: knockout; MEALAS: mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; nf: necrotic core-free; NMO: neuromuscular organoid; SCO: spinal cord organoid; SMA: spinal muscular atrophy; VPA: valproic acid; ZO: zonula occludens.

spinal cord, leading to significant neuronal damage. This system can be used for screening neuroprotective agents. The neural tube development of a fetus can be affected by genetic factors or pharmacological stimuli, potentially leading to delayed neuronal development and issues such as ischaemia and hypoxia. In 2023, researchers developed hSCOs, and observed that valproic acid treatment can induce neurodevelopmental defects in the organoid model, including impaired expression of tight junction proteins zonula occludens 1 and 2,[43] providing a suitable research model for the study of human neural tube defect diseases. Zou et al.[44] employed hCOs and hSCOs to study the function of fused in sarcoma (FUS) in neurodevelopment. They found that FUS-knockout hSCOs exhibited impaired motor neuron development, uncovering a role for FUS in regulating Ntrk3 (neurotrophin receptor kinase 3) expression and neural development. Furthermore, organoids serve as a tool to investigate the neurotoxic effects of chemicals that induce fetal developmental defects. Lee et al.[45] used SCOs to study the impact of varying concentrations of antiepileptic drugs on neurotube morphogenesis, demonstrating a dose-dependent effect of valproic acid and carbamazepine on neurotube closure defects. Lundin et al.[52] developed the RosetteArray® platform, which allows for the generation of over 9000 micropatterned 3D forebrain and SCOs on a single 96-well plate, each with the characteristic of singularly polarised neural rosette tissues. By utilising artificial intelligence technology to recognise rosette morphologies, this platform enables high-throughput screening for small molecules that pose risks for neurodevelopmental disorders and potential therapeutics, providing a high-throughput and precise research platform for related diseases.

MELAS, which stands for mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes, is a multi-system metabolic disorder stemming from mutations in either mitochondrial DNA or nuclear DNA, typically inherited maternally. This condition often affects the nervous system and muscles, leading to an accumulation of lactic acid in the body and potentially causing recurrent episodes akin to strokes.[46] Winanto et al.[46] utilised iPSC-derived hSCOs from MEALAS patients to investigate the role of Notch signalling in neural development associated with MEALAS. This model revealed that elevated Notch signalling was responsible for the delayed motor neuron development and axonal growth defects observed in MEALAS. The study also demonstrated that treatment with the γ-secretase inhibitor N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester could reverse these neurodevelopmental deficits.

Neurodegenerative disease

Neurodegenerative diseases associated with spinal cord tissue are often caused by genetic defects, age-related, with the structure and function of the spinal cord gradually deteriorating over time, thereby affecting the transmission of neural signals and a variety of bodily functions. Degenerative conditions of the spinal cord include amyotrophic lateral sclerosis (ALS) and multiple sclerosis. ALS is a progressive neurodegenerative disease that affects motor neurons in CNS. As ALS progresses, motor neurons degenerated, leading to muscle weakness, twitching, and eventually, atrophy. Gradually, the ability of the brain to control muscle movement is lost, and patients may become completely paralysed with an average survival time of 3–5 years after diagnosis. Although genetic factors play an important role, the cause of ALS is not fully understood and there is currently no cure for ALS. Research into ALS is ongoing, aiming at understanding the genetic and environmental factors that contribute to the disease, developing new treatments, and ultimately finding a cure. Multiple sclerosis is a demyelinating disease of the CNS, leading to inflammation, demyelination, and axonal damage that disrupts the transmission of neural

signals, thereby affecting sensory perception, motor function, and coordination. The pathogenesis of this disease remains incompletely elucidated, with genetic factors, environmental influences, and immune system anomalies all contributing to disease progression.[53-56] Currently, researchers have utilised organoid models to identify mutations in specific genes that play a significant role in the development of the disease.[57-59]

Gao et al.[47] have developed neuromuscular organoids that encompass both trunk spinal cord neurons and peripheral muscle systems. By utilising iPSCs harbouring the *C9orf72* mutation from patients with ALS, they constructed an *in vitro* ALS organoid model that recapitulates key pathological features of the disease, including muscular dystrophy and loss of Schwann cells. The authors also employed this model to test the effects of the small molecule inhibitor GSK2606414 on ALS organoids, providing a suitable organoid system for the study of spinal neuromuscular pathology and its application in drug testing. Additionally, Guo et al.[59] investigated the establishment of neuronal cell types from human iPSCs with *C9orf72* knockdown and generated SCOs to model ALS, uncovering ALS pathological characteristics of neuroinflammation in this model. Sirtori et al.[60] also explored the role of the *C9orf72* mutation in ALS pathogenesis using SCO models, discovering that disruption of the linker of nucleoskeleton and cytoskeleton complex within the nuclear envelope leads to alterations in nuclear morphology of neurons within the organoids, elucidating a novel mechanism in the pathogenesis of ALS.

Viral-induced neurological disorders

Organoids frequently serve as effective 3D models for studying human viral infections. Since their inception, CNS organoids have been extensively utilised in viral pathogenesis research, including the investigation of neural degeneration induced by coronavirus disease 2019.[61, 62] In the context of spinal cord-related viral pathologies, enterovirus D68 is known to cause severe acute flaccid myelitis associated with respiratory illness, leading to paralysis in children. By employing two strains of enterovirus D68 to infect hSCOs that encompass motor neurons, interneurons, and glial cells, researchers have discovered that the echovirus 11 strain induces significant organoid structural disruption and cellular apoptosis.[48] This finding underscores the pivotal role of organoid models in unraveling the intricate mechanisms of viral-induced neurological disorders.

Spinal cord injury and neuromuscular diseases

SCI is a serious CNS injury, often resulting from trauma or compression to the spinal cord, leading to varying degrees of motor and sensory dysfunction.[63] The severity and location of the SCI determine the extent of neurological deficit, causing autonomic dysregulation, permanent muscle paralysis, sensory loss or abnormalities, and affecting bladder and bowel function. SCI serves as a crucial disease model for studying neural injury and functional recovery. To date, achieving functional restoration after SCI remains challenging. Prior to the advent of organoid technology, stem cell transplantation was considered a cutting-edge and focal therapy for SCI. However, the low survival rate of transplanted cells, uncontrollable differentiation and tumorigenic potential have limited the clinical application of this technology. Recently, researchers have reprogrammed human astrocytes into early neural ectoderm cells, generating SCOs with dorsal and ventral specific neuronal subtypes. When transplanted into a complete transection SCI model, these organoids bridge the injured tissue of the host.[64] This study offers a potential therapeutic approach for SCI by utilising a delivery system to achieve in situ reprogramming of glial scars at the site of injury, forming functional spinal cord tissue, thus avoiding invasive surgery.

Despite advancements in organoid technology, there is currently a lack of SCOs that can fully simulate the pathological processes of SCI. The trajectory of traumatic SCI encompasses both primary and secondary injuries.[65, 66] Acute SCI typically results from fractures and dislocations of the vertebral column. The initial phase of injury is termed the primary injury, where bone fragments and ligamentous tearing due to spinal trauma cause structural damage to the spinal cord. This phase is characterised by haemorrhage, destruction of neural substance, and damage to axonal networks. Secondary injury changes include apoptotic signalling, vascular damage, ischaemia, excitotoxicity, ion imbalance, inflammation, free radical formation, demyelination, fibroglial scarring, and cyst formation. Although ultrasound stimulation has been used to construct an *in vitro* model of mechanical injury based on COs for analysing the pathological mechanisms of traumatic brain injury,[67] the complex pathological microenvironment of SCI remains challenging to mimic in organoid models due to the absence of immune cell infiltration.

Spinal muscular atrophy (SMA) is a hereditary neuromuscular disorder characterised by the degeneration of motor neurons in the spinal cord, leading to muscle weakness and atrophy. Grass et al.[49] utilised iPSCs to generate SCOs that model the early state of SMA. These organoids exhibit aberrant morphological development, a reduction in the expression of neuronal progenitor markers, and a significant deficiency in motor neuron numbers. There is a developmental bias towards mesodermal progenitors and muscle cells. The phenotypes can also be observed in SMA mouse embryos. Interestingly, the conversion from SMN (survival motor neuron gene) 2 to SMN1 did not fully reverse these developmental irregularities, suggesting that early neurodevelopmental deficits may underlie the later motor neuron degeneration in SMA. This work uncovers the role of the *SMN* gene in neuronal progenitor and mesodermal differentiation.

Pain

Dorsal root ganglia (DRGs) within spinal cord tissue contain a plethora of neuronal nuclei and serve as crucial relay stations for the conduction of pain signals, making them a focal point in clinical research for alleviating neuropathic pain. However, the difficulty in obtaining human DRG tissue has impeded preclinical drug screening studies.[68, 69] In 2020, researchers developed DRG organoids derived from fibroblasts, which exhibit cellular composition and molecular characteristics akin to *in vivo* DRG tissue.[70] Recently, investigators have also engineered SCO-on-chip to replicate the nociceptive neural circuits. This research validated the feasibility of organoid-on-chip for pain research by subjecting them to physically

and chemically induced painful conditions and modulating nociceptive molecules.[50]

Despite the development of various disease models based on SCOs, they still have certain limitations compared to other models. Vascularisation of SCOs remains a challenge, leading to insufficient local oxygen and nutrient supply, which may result in necrosis at the centre of the SCOs, limiting the scale and function of the models.[71] The maturity neurons in SCOs obtained *in vitro* is generally low, and their electrophysiological functions may still not be as advanced as the spinal cord tissue in animal models.[72] Although SCOs can mimic certain disease states, replicating the complex pathological characteristics of spinal cord diseases is still challenging.

In summary, SCOs provide a powerful platform for studying spinal cord development and disease research, but further technical improvements and optimisations are needed to overcome the existing limitations. With the advancement of bioengineering and stem cell technology, it is anticipated that SCOs will play an increasingly important role in the study of spinal cord diseases.

Spinal Cord Organoid Construction by Bioengineering

Although the aforementioned researches have generated SCOs capable of representing certain cellular compositions at a specific developmental stage of spinal cord tissue, these organoids, which are self-organised from pluripotent stem cells within an induction system, have yet to achieve an accurate replication of the spinal cord's structure. This encompasses the detailed replication of the functional cellular composition, spatial distribution, segmental organisation, and D-V developmental characteristics. Advanced techniques, including bioactive materials, 3D printing, micropatterning, and microfluidic chips, have been implemented in SCO research to facilitate the meticulous construction of spinal cord tissue structures. Moreover, researchers have also developed "assembloids" to investigate the intricacies of cellular interactions and the formation of neural circuits.

Biomaterials and three-dimensional printing

The construction of SCOs based on functional biomaterials has garnered attention in recent years. Biomaterials can provide an adjustable microenvironment to guide cell fate in organoids. The biochemical and mechanical properties of the extracellular matrix (ECM) have a significant impact on the biological behaviour of neural cells, such as axonal projection and synapse formation. Therefore, the characteristics of biomaterials used in the construction of neural organoids can influence the morphology and cellular functions of the organoids.[73, 74] In 2017, Lancaster et al.[75] utilised poly(lactic-co-glycolic acid) microfilaments as a scaffold for the growth of iPSCs to physically direct cellular polarisation. In concert with Matrigel and neural induction medium, they obtained elongated embryoid bodies that enhanced the formation of the neural ectoderm. Functional biomaterials can adjust tissue size and geometric shape, further influencing cell-cell signalling pathways.

A variety of biomaterials have been extensively studied for the construction of SCOs, each offering unique advantages and properties that facilitate the mimicry of the spinal cord's complex microenvironment (**Table 2**).[58, 74, 76-82] One of the commonly utilised biomaterials is Matrigel, a commercially available ECM extract that support the growth and differentiation of various cell types. Synthetic hydrogels, such as poly(lactic-co-glycolic acid),[75] polyethylene glycol,[83] and polyglycolic acid.[84] These materials can guide the adhesion and migration of neural progenitor cells and promote axonal regeneration. Hyaluronic acid hydrogels have shown significant potential in the application of human neural organoid generation, due to the high content of hyaluronic acid in the ECM of the human CNS.[85] Self-assembling peptides and recombinant proteins possess specific rheological, mechanical, and chemical properties, and their degradation rates can be programmed by introducing protease recognition sites or altering cross-linking chemistry. Photo-curing materials have enabled the precise fabrication of complex geometric bio-scaffolds.[86] These materials are instrumental in constructing SCOs and facilitating their integration with host tissues. Composite scaffolds combine the advantages of different materials, such as the combination of chitosan microspheres with Matrigel for spatially specific delivery of Shh agonists, mimicking the centre of *in vivo* spinal tissue and generating distinct D-V progenitor domains.[74]

Matrigel is commonly employed as a coating material for the generation of neural organoids.[6, 87, 88] Matrigel is a temperature-sensitive matrix rich in components such as collagen IV, laminin, glycosaminoglycans, and growth factors, which derived from mouse sarcoma cells.[6] Although Matrigel provides an appropriate ECM signalling for organoid growth and differentiation, the unclear composition prevents researchers from pinpointing the key signals associated with cell proliferation and differentiation. Moreover, the animal-derived components of Matrigel can affect the uniformity of organoid development across different batches.[89] Additionally, Matrigel may elicit immune rejection responses when it comes to clinical transplantation applications. In contrast, synthetic hydrogels offer a more defined composition and can be designed to direct cell differentiation.[58, 90] In recent work, Chooi et al.[58] employed alginate hydrogels as an alternative to Matrigel for the construction of SCOs. They found that alginate hydrogel-supported SCOs exhibited similar efficiencies in neuronal and glial cell differentiation as those encapsulated in Matrigel. Compared to Matrigel, SCOs based on alginate hydrogels displayed less inter-individual variability. Furthermore, this synthetic hydrogel better regulated the fate of neural cells, reducing the differentiation of non-spinal cord characteristic cells within the organoids. Utilising alginate hydrogel-based SCOs, they established a disease model for ALS with a *TDP43* (G298S) mutation, and observed an increase in the mislocalisation of TDP43 within the mutant organoids. Li et al.[76] found that organoid encapsulation using microgels and nanogels, as a strategy of cell surface engineering, offers controllable permeability to different molecules. Controlling the permeability of tumour necrosis factor-α can inhibit apoptosis under adverse conditions, making the development of such gels significant for organoid transplantation therapies.

Table 2. Biomaterials applied in generation of SCOs

Type	Material	Cell	Application	Advancement/insight	Reference
Hydrogel	Alginate	iPSCs	ALS SCOs model with a *TDP43* (G298S) mutation	Produce a xeno-free and fully defined 3D culture condition for organoid generation	58
Nanogel	HA	PC12 cells	Applied for the encapsulation on individual neuronal cell	Controlling the permeability of TNF-α, inhibit apoptosis under adverse conditions	76
ECM hydrogel	Decellularised brain ECM	iPSCs	Advance the development of SCOs	SCOs generated more mature neurons, and exhibit higher levels of markers for multiple compartments of the native spinal cord	77
ECM hydrogel	Decellularised spinal cord ECM	NPCs	Act as a delivery vehicle for NPCs and organoids in SCI models	The ECM derived from neonatal rabbit spinal cord tissue has superior potential to promote the proliferation, migration, and differentiation of neural progenitors	78
ECM hydrogel	Decellularised placenta-derived ECM	iPSCs	Accelerate the developmental process of SCOs	SCOs derived more mature cellular phenotypes, can be applied in future personalised medicine	79
PCSM-Matrigel@SAG	Chitosan microspheres combined with Matrigel	iPSCs	Spatially regulate the concentration distribution of Shh signal	Generate the D-V-like cytoarchitecture with domain-specific progenitors and neurons.	74
Bioink	Gelatin cross-linked by mTG	BC- and iPSC-derived neural cells	3D printing	The 3D bioprinted scaffold is suitable for the survival and differentiation of human neural cells	80
Scaffold material	Collagen sponge	NSCs and OPCs	Bioengineer the spinal cord-like structure	Act as scaffolds for SCLT with white matter and gray matter-like structure	81, 82

Note: 3D: three-dimensional;ALS: amyotrophic lateral sclerosis; BC: boundary cap neural crest stem cells; D-V: dorsal-ventral; ECM: extracellular matrix; HA: hyaluronic acid; iPSCs: induced pluripotent stem cells; mTG: microtransglutaminase; NPCs: neural progenitor cells; NSCs: neural stem cells; OPCs: oligodendrocyte precursor cells; PC12: pheochromocytoma cell; PCSM: porous chitosan microsphere; SAG: Shh agonist agonist; SCI: spinal cord injury; SCLT: spinal cord like tissue; SCO: spinal cord organoid; TDP43: TAR DNA-binding protein 43; TNF: tumour necrosis factor.

Decellularised ECM components from neural tissues are an excellent choice for synthetic hydrogels used in organoid. The ECM of spinal cord tissue primarily comprises fibronectin, collagen IV, laminin, and proteoglycans. These extracts can be solubilised by modulating temperature and pH, followed by reorganisation of intramolecular bonds to form synthetic hydrogels. Tissue-specific extracellular matrices are key drivers in the development of complex organs. These prepared gels support cell growth, differentiation, and neurite extension, showing great promise in neural organoid technology (**Figure 5**).[90-92] In 2024, Wu et al.[77] developed a rat decellularised brain ECM hydrogel that supports the formation of hiPSC-derived SCOs. Compared to Matrigel, organoids cultured in decellularised brain ECM hydrogel exhibit higher levels of markers for multiple compartments of the native spinal cord, facilitating the maturation of neurons. In the same year, Sun et al.[78] utilised decellularised spinal cord ECM to construct a SCO model and found that the matrix derived from neonatal rabbit spinal cord tissue has superior potential to promote the proliferation, migration, and differentiation of neural progenitors compared to that from adult rabbits, identifying pleiotrophin and tenascin as the proteins playing a role. Wang et al.[79] extracted a human decellularised placenta-derived ECM hydrogel for SCO construction, and achieved more mature cellular phenotypes, indicating the application prospects of human extracellular matrices in personalised medicine.

Studies have demonstrated that the concentration, location, and timing of signalling pathway ligands determine cellular fate, and the use of biomaterials allows for precise control over these factors. In 2014, a study utilised a laminin and polyethylene glycol hydrogel scaffold to control release of RA, inducing the formation of neuroepithelial tissue with a D-V structure.[15] Recently, researchers employed porous chitosan microspheres combined with Matrigel to spatially regulate the concentration distribution of Shh agonists. This approach mimics the signalling centres of neural tube development *in vivo* and generates distinct dorsal and ventral progenitor domains.[74] The combination of biomaterials with 3D printing technology can be used to shape organoid morphology and design the spatial distribution of cells and inductive factors. Han et al.[80] used gelatin-based bioink, with microtransglutaminase as a cross-linking agent, to identify the optimal 3D bioprinted scaffold suitable for the survival and differentiation of boundary cap neural crest stem cells and iPSC-derived neural cells (motor neurons and astrocytes), offering new insights for the bioprinting of multicellular SCOs.

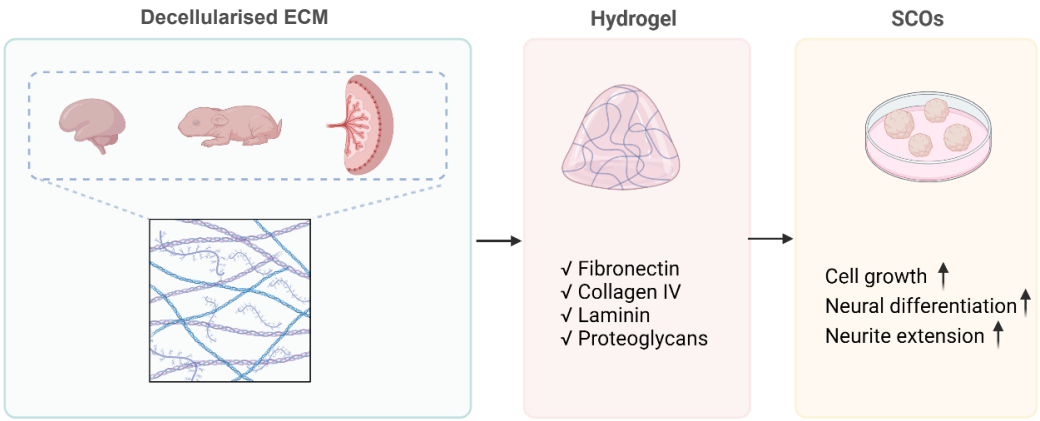

Figure 5. Decellularised ECM hydrogels, derived from sources such as rat brain tissue, neonatal rabbit spinal cord tissue, and human placental tissue, are increasingly utilised in the construction of SCOs. These hydrogels enhance the maturation and functionality of neural cells within SCOs, presenting a significant advancement for research into pathogenic genes and the screening of small molecule therapeutics. Created with BioRender.com. ECM: extracellular matrix; SCOs: spinal cord organoids.

SCO generation is inseparable from the developmental microenvironment. Smart biomaterials can adjust the physical or biochemical characteristics, have been used in numerous applications, including drug delivery and dynamic mechanobiology.[93] They are suitable choice for simulating dynamic changes in human body and promoting cell differentiation. Meanwhile, immunological rejection and inflammation are key factors limiting clinical applications of SCOs transplantation. Developing functional carriers with biomaterials is promising to protect organoids from the host immune system upon transplantation.[78] Hydrogels are the most widely used scaffold materials, with the advantages of mild synthesis and high suitability for organoid transplantation.[94] To minimise immune rejection, not only the iPSCs can be derived from the patient, but also the biomaterials can be extracted from human tissues for personalised medicine design, enhancing their clinical application prospects. In 2022, researchers discovered that cells and hydrogels from the same individual exhibit synergistic effects in mimicking the formation of embryonic spinal cord.[95]

Although biomaterials show great potential in the application of SCOs, there are still challenges to overcome, such as permitting cell adhesion, preventing neural apoptosis, controlling the degradation rate of the materials, and avoiding biological toxicity.[96] Hydrogels also might have the limitation of poor permeability or inappropriate viscoelasticity.[97] Thus, synthetic biomaterials need further optimising to ensure nutrients and metabolites delivery of organoids. The efficiency and biocompatibility of novel biomaterials require further validation, but they undoubtedly represent a promising direction in SCO research.

In addition to modulating the physicochemical signals related to cell fate, biomaterials possess an innate advantage for the regionalised and precise design of spinal cord-like structures.[98] Researchers have designed various spinal cord-like tissues (SCLTs) by adjusting the mechanical properties of biomaterials, loading small molecules, and combining them with NSCs. SCLTs resemble natural spinal cord in tissue morphology, holding immense potential for reconstructing damaged spinal cord neural networks. In 2018, researchers simulated the white and gray matter of the spinal cord using tissue engineering to construct transplantable SCLTs based on gelatin sponge and NSCs (**Figure 6A**), the SLCTs shows the white matter and gray mater like structure (**Figure 6B–D**). Within these SCLTs, they observed myelin formation and neuronal electrophysiological activity, and SCLTs were able to establish signal transmission with the DRG and muscle cells in rats.[81] When the research team combined SCLT transplantation with caudal nerve electrical stimulation, the therapeutic effect was further enhanced, including Basso-Beattie-Bresnahan score and motor evoked potential improvement after transplantation (**Figure 6G**, and **H**).[82]

Biomaterials can also be integrated with other cutting-edge technologies to more effectively regulate the structure and function of organoids. Two-photon technology has been employed to control the spatial distribution of neurotrophic factors, guiding the growth of neuronal axons during organoid morphogenesis; additionally, novel synthetic protein hybrid materials or 3D printing techniques are used to simulate the distribution patterns of growth factors, directing angiogenesis within organoids and more accurately recapitulating the morphogenesis of the CNS.[99-101] In 2023, a study developed a hydrogel-in-hydrogel 3D printing method capable of dynamically adjusting hydrogel components within pre-existing hydrogel-based organoid cultures. This technique achieves the adjustment of hydrogel geometry and mechanical properties through two-photon absorption cross-linking of photosensitive hydrogels during the culture process. The study found that the combination of two-photon and 3D printing to modulate hydrogel properties can influence cell migration during organoid development, and hydrogels obtained through this technology can guide the directional growth of neuronal axons in SCLTs.[102]

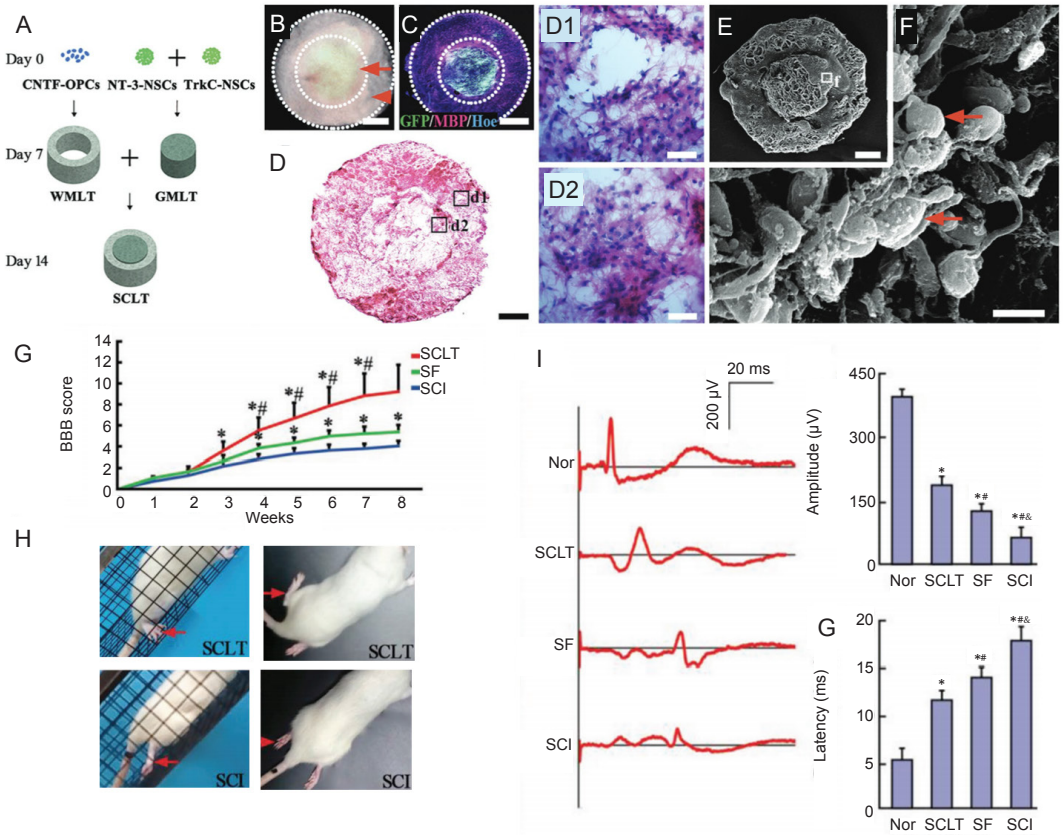

Figure 6. Assembly methodology of the SCLT and its application in SCI. (A) Fabrication of engineered hydrogels and assembly with OPCs and NSCs. (B–D) Brightfield morphology of SCLT and cellular morphology revealed by HE staining. The arrows indicate the GMLT and WMLT structure in SCLT. Scale bars: 500 μm (B–D), 40 μm (D1, D2). (E, F) SEM appearance of SCLT after 14 days of culture. The arrows indicate the cell bodies in SCLT. Scale bar: 500 μm (E) and 5 μm (F). (G, H) Improvement in hindlimb motor function in rats post-SCLT transplantation. The arrows indicate the different postures of the hind limbs while in motion in each treatment group. (I) Significant enhancement in MEP in animals following SCLT transplantation, indicating neural conductivity recovery. *P < 0.05, *vs.* Nor group; #P < 0.05, *vs.* SCLT group; &P < 0.05, *vs.* SF group. Reprinted from Lai et al.[81] BBB: Basso-Beattie-Bresnahan; CNTF: ciliary neurotrophic factor; GFP; green fluorescent protein; GMLT: gray matter like tissue; Hoe: Hoechst; MBP: myelin basic protein; MEP: motor evoked potential; Nor: normal; NT-3: neurotrophin-3; NSC: neural stem cell; OPCs: oligodendrocyte precursor cells; SCI: spinal cord injury; SCLT: spinal cord like tissue; SEM: scanning electron microscopy; SF: scaffold; WMLT: white matter like tissue.

Organoid engineered by micropattern and microfluidic chip

Previous studies have indicated that the abundant presence of neurorosettes in SCOs disrupts the morphogenesis of the neural tube, ultimately hindering tissue maturation and affecting the efficiency of organoid formation.[6, 103] In 2018, Knight et al.[103] patterned the tissue morphology of organoids by micropatterning, using iPSCs to induce neural organoids with a singular neurorosette structure, which could be maintained throughout subsequent tissue morphogenesis. Additionally, micropatterning is an efficient method for generating heterogeneity in the body axis regions of the neural tube. By controlling the spatiotemporal gradients of morphogens, it precisely regulates stem cell fate, mimicking the formation of A-P and D-V axes during neural development. Seo et al.[104] demonstrated that micropatterning could influence the ratio of D-V domains in SCOs by altering the size of the micropatterns, thereby controlling the proportion of centre-edge cells. In 2023, research reported a bioengineering and machine learning framework that optimises the asymmetric distribution of organoid developmental signals by adjusting the spatial coupling of micropattern-seeded cells, resulting in organoids that elongate along the A-P axis. Through the use of fibroblast growth factor signalling and secreted Wnt inhibitors, neural tube-like structures with distinct neural cell types were obtained, including axial progenitors, spinal cord precursors, hindbrain, and forebrain regions.[105] Xue et al.[106] reported a microfluidic neural tube-like structure, combining micro pattern technology to recapitulate the formation of neural patterns in brain and spinal cord regions along the rostral-caudal and D–V axes (**Figure** 7). In this model, researchers observed the formation of neural crest cells, intact neural tubes, and forebrain-like structures, achieving a comprehensive neural tube-like tissue. This approach allows researchers to precisely control cell spatial arrangement and differentiation, providing a powerful tool for studying neural development and disease models.

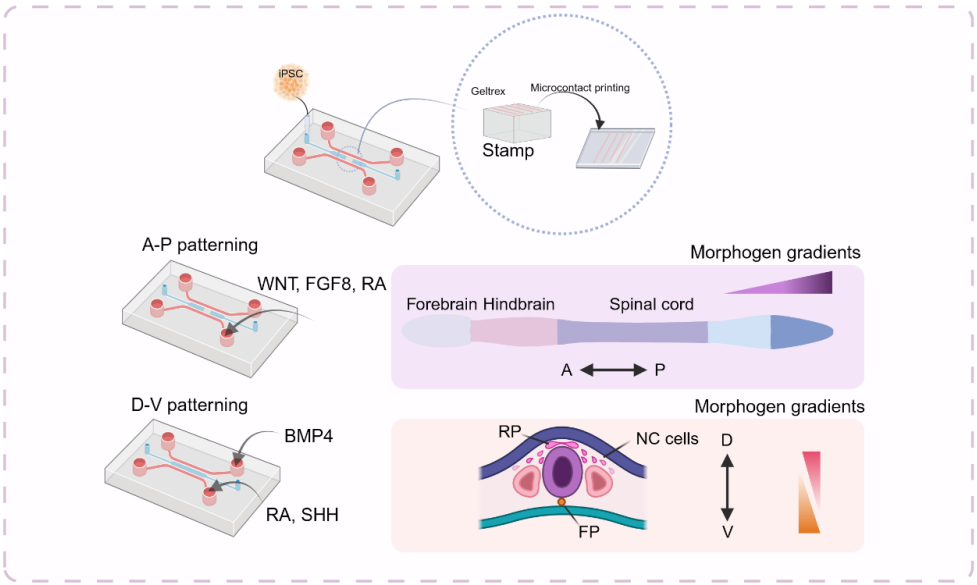

Figure 7. Schematic representation of the process for obtaining microfluidic neural tube-like structure utilising micropatterning and microfluidic techniques. By simulating the morphogen gradients in the body, the cell fate of organoids-on-chip is regulated, leading to the development of organoids that mimic early neural tube morphogenesis, including the acquisition of NC cells. Created with BioRender.com. A-P: anterior-posterior; BMP: bone morphogenetic protein; D-V: dorsal-ventral; FGF: fibroblast growth factor; FP: floor plate; iPSC: induced pluripotent stem cell; NC: neural crest; RA: retinoic acid; RP: roof plate; SHH: sonic hedgehog.

Assembloids

The white matter of the spinal cord encompasses numerous ascending and descending nerve fibres that link the brain with the motor and sensory units of the peripheral nervous system. The integration of organoid, named "Assembloid", offers a novel approach to studying these intricate intercellular interactions and neural circuits (**Figure 8**). The fusion of organoid techniques entails the amalgamation of diverse organ models to simulate the entire neural network connecting the brain, spinal cord, and target muscle groups *in vitro*, providing a comprehensive model for the assessment of neural function and in-depth investigation of complex disease models. Giandomenico et al.[3] generated cortical organoids with enhanced activity using air-liquid interface culture, which established connections with mouse embryonic spinal cord, eliciting contractions in adjacent muscles. In 2020, two studies fused SCOs with COs to form assembloids, which were subsequently integrated with skeletal muscle precursors to create cortico-spinal muscular organoids.[107, 108] Physical or chemical stimulation of the COs within these assembloids induced contractions in muscle tissue, demonstrating the functional connectivity. Son et al.[109] developed an engineered brain-spinal cord assembloid by co-culturing COs with motor neuron spheres (MNSs). They utilised a 3D engineered chip to connect MNSs to specific regions of COs, mimicking the physiological connections between the cortex and the spinal cord. The assembloid could transmit caffeine-induced stimulation from COs to MNSs, increasing the firing rate of MNS neurons, making the engineered brain-spinal cord assembloid system a screening platform for validating the transmission of neurochemical stimuli. Hong et al.[110] stably expressed the light-sensitive protein channelrhodopsin-2 in COs, connecting them with SCOs and muscle spheres. Through optogenetic stimulation of the COs, they successfully induced strong and consistent contractions in muscle cells.

Outlook and Perspectives

To date, SCOs have been capable of mimicking the early developmental processes of the spinal cord, replicating the initial stages of neural tube formation. Concurrently, advancements have been made in the developmental patterning, and functional research of SCOs. Numerous study have generated organoids with specific functional cells and gene expression patterns by modulating inductive factors.[22] However, existing SCOs still have many limitations that hinder their application as disease models and grafts. The first limitation is the lack of cellular diversity within the spinal cord. Previous studies on SCOs have primarily focused on motor neurons,[64, 111] with a deficiency in the integration of sensory neurons and microglia, leaving a gap in SCO models for disorders such as neuropathic pain and neuroinflammation. Furthermore, due to the difficulty in balancing differentiation efficiency and phenotypic diversity, establishing an *in vitro* model with a variety of mature neuronal subtypes to replicate the morphological development of the spinal cord remains challenging. The absence of vasculature in SCOs and the limitations of developmental regulatory factors *in vitro* mean that the organoids currently obtain can only simulate the early developmental stages of the neural tube. Faced with the limitations, incorporating mesenchymal cells, inflammatory cells into SCOs might improve their maturity and

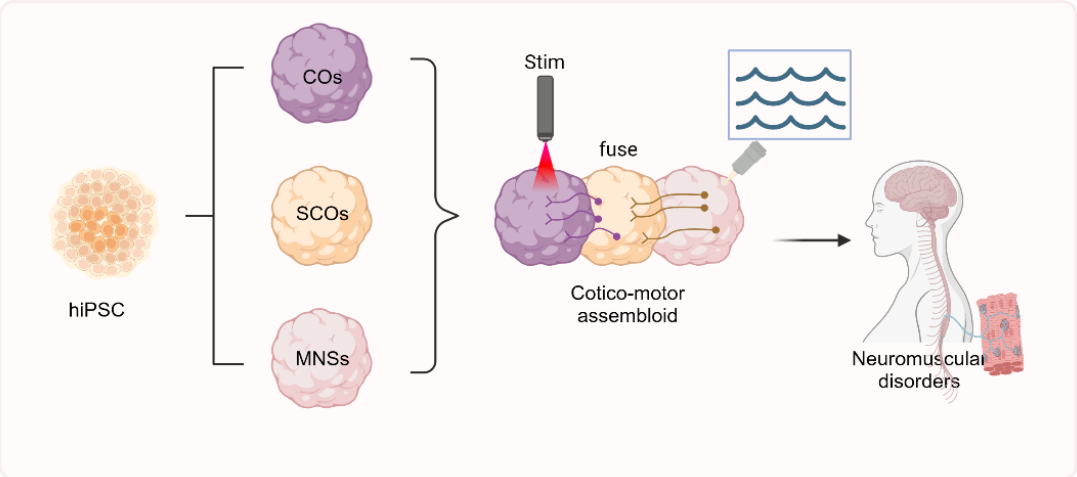

Figure 8. Method for obtaining assembloids and their applications. By integrating COs, SCOs, and MNSs derived from hiPSCs, cotico-motor assembloids are generated. Stimulation of the COs module within the assembloid can receive muscle contraction signals detected on the MNSs. Assembloids are utilizable for investigating neuromuscular signal transmission and the pathological mechanisms of related diseases. Created with BioRender.com. COs: cortical organoids; hiPSCs: human induced pluripotent stem cells; MNSs: motor neuron spheroids; SCOs: spinal cord organoids; Stim: stimulation.

create functional microtissues to more accurately recapitulate neurodevelopment of spinal cord.[112] Vascularisation via SCOs coculture with endothelial cells could increase nutrient exchange in a near-physiological way, alleviating the limitation of SCOs sizes. Additionally, pretreatment of material carrier with small molecule compounds might be a feasible solution to lagged maturation, providing possibilities for disease modelling in later developmental stages.[113] There is still a long way to go before they can be widely applied in clinical translation. Additionally, the application of bioelectricity may effectively regulate neural development, stimulate neuronal maturation, and yield functionally enhanced SCOs.[114] In terms of tissue structure simulation of the spinal cord, current research focuses on A-P and D-V morphogenesis, while M-L axis distribution of cell bodies and neural fibres is still insufficient. Further improving the precision of 3D printing technology and the spatiotemporal distribution of neural inductive factors is expected to address this challenge.

Limitations

This review on SCO research, while providing a detailed analysis of the current state of the field, is not without its limitations. Firstly, the scope of literature coverage may be constrained by the specific databases and search terms utilised, potentially omitting significant studies in emerging or specialised areas of SCO research. This could lead to a less comprehensive overview of the existing knowledge and the nuances of methodological advancements. Secondly, the rapid evolution of SCO technology poses a challenge for the review's updatability. Given the swift pace of research in this domain, the articles included in the review may not encompass the most recent breakthroughs, thus affecting the timeliness and currency of the information presented. This delay could impact the review's ability to accurately reflect the cutting-edge status of SCO research and its applications. Lastly, the review may suffer from a paucity of clinical data regarding the application of SCOs in neural regeneration. The lack of robust data on the clinical efficacy and safety of SCOs could hinder the review's capacity to thoroughly assess the technology's practical implications and its potential future trajectory in the realm of regenerative medicine. In conclusion, while the review offers insights into the progress and potential of SCO research, it is essential to consider these limitations when interpreting its findings and projections. Ongoing efforts to broaden the scope of literature reviews, incorporate the latest research, and amplify clinical data collection are crucial for maintaining the relevance and reliability of such reviews in the dynamic field of SCO research.

Conclusion

SCOs are propelling the development of medical treatment strategies for CNS diseases. They serve not only as models for disease and developmental research but also as grafts, integrating with the host's damaged tissue to facilitate therapeutic functional restoration.[112] SCOs also act as disease models for studying pathogenic genes of the spinal cord, utilising patient-derived iPSCs or cell lines with known pathogenic gene defects. Significant research progress has been made in the study of neurodevelopmental disorders, neuropathic pain, neurodegenerative diseases, and SCI. Concurrently, biofunctional materials, 3D printing technology, organ-on-a-chip technology, and assembloids have aided in refining the structure and function of SCOs. However, organoid of next generation needs to focus on addressing issues such as cellular maturity, diversity, and structural biomimesis. We anticipate that in the future, SCOs will be widely applied in clinical settings for disease screening and will further extend their applications in transplantation and tissue regeneration.

Author contributions

Conceptualisation: RZ; data collection, literature reviewing, and manuscriptdrafting: RH, YZ, LYu, ZL, YL and ZW; reviewing and editing: HC, XH, LYang, XX, YB and BC. All authors read and approved the final version of the manuscript.

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Conflicts of interest statement

The authors declare no competing financial interest.

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1. Lancaster, M. A.; Knoblich, J. A. Organogenesis in a dish: modeling development and disease using organoid technologies. *Science.* **2014,** *345,* 1247125.
2. Bian, S.; Repic, M.; Guo, Z.; Kavirayani, A.; Burkard, T.; Bagley, J. A.; Krauditsch, C.; Knoblich, J. A. Genetically engineered cerebral organoids model brain tumor formation. *Nat Methods.* **2018,** *15,* 631-639.
3. Giandomenico, S. L.; Mierau, S. B.; Gibbons, G. M.; Wenger, L. M. D.; Masullo, L.; Sit, T.; Sutcliffe, M.; Boulanger, J.; Tripodi, M.; Derivery, E.; Paulsen, O.; Lakatos, A.; Lancaster, M. A. Cerebral organoids at the air-liquid interface generate diverse nerve tracts with functional output. *Nat Neurosci.* **2019,** *22,* 669-679.
4. Cenini, G.; Hebisch, M.; Iefremova, V.; Flitsch, L. J.; Breitkreuz, Y.; Tanzi, R. E.; Kim, D. Y.; Peitz, M.; Brüstle, O. Dissecting Alzheimer's disease pathogenesis in human 2D and 3D models. *Mol Cell Neurosci.* **2021,** *110,* 103568.
5. Zhou, G.; Pang, S.; Li, Y.; Gao, J. Progress in the generation of spinal cord organoids over the past decade and future perspectives. *Neural Regen Res.* **2024,** *19,* 1013-1019.
6. Lancaster, M. A.; Renner, M.; Martin, C. A.; Wenzel, D.; Bicknell, L. S.; Hurles, M. E.; Homfray, T.; Penninger, J. M.; Jackson, A. P.; Knoblich, J. A. Cerebral organoids model human brain development and microcephaly. *Nature.* **2013,** *501,* 373-379.
7. Zhou, C.; Wu, Y.; Wang, Z.; Liu, Y.; Yu, J.; Wang, W.; Chen, S.; Wu, W.; Wang, J.; Qian, G.; He, A. Standardization of organoid culture in cancer research. *Cancer Med.* **2023,** *12,* 14375-14386.
8. Smirnova, L.; Hartung, T. The promise and potential of brain organoids. *Adv Healthc Mater.* **2024,** *13,* e2302745.
9. Mulder, L. A.; Depla, J. A.; Sridhar, A.; Wolthers, K.; Pajkrt, D.; Vieira de Sá, R. A beginner's guide on the use of brain organoids for neuroscientists: a systematic review. *Stem Cell Res Ther.* **2023,** *14,* 87.
10. Sagner, A.; Briscoe, J. Establishing neuronal diversity in the spinal cord: a time and a place. *Development.* **2019,** *146,* dev182154.
11. Carpenter, E. M. Hox genes and spinal cord development. *Dev Neurosci.* **2002,** *24,* 24-34.
12. Al Oustah, A.; Danesin, C.; Khouri-Farah, N.; Farreny, M. A.; Escalas, N.; Cochard, P.; Glise, B.; Soula, C. Dynamics of sonic hedgehog signaling in the ventral spinal cord are controlled by intrinsic changes in source cells requiring sulfatase 1. *Development.* **2014,** *141,* 1392-1403.
13. Song, L.; Liu, Y.; Yu, Y.; Duan, X.; Qi, S.; Liu, Y. Shh signaling guides spatial pathfinding of raphespinal tract axons by multidirectional repulsion. *Cell Res.* **2012,** *22,* 697-716.
14. Lippmann, E. S.; Williams, C. E.; Ruhl, D. A.; Estevez-Silva, M. C.; Chapman, E. R.; Coon, J. J.; Ashton, R. S. Deterministic HOX patterning in human pluripotent stem cell-derived neuroectoderm. *Stem Cell Reports.* **2015,** *4,* 632-644.
15. Meinhardt, A.; Eberle, D.; Tazaki, A.; Ranga, A.; Niesche, M.; Wilsch-Bräuninger, M.; Stec, A.; Schackert, G.; Lutolf, M.; Tanaka, E. M. 3D reconstitution of the patterned neural tube from embryonic stem cells. *Stem Cell Reports.* **2014,** *3,* 987-999.
16. Ranga, A.; Girgin, M.; Meinhardt, A.; Eberle, D.; Caiazzo, M.; Tanaka, E. M.; Lutolf, M. P. Neural tube morphogenesis in synthetic 3D microenvironments. *Proc Natl Acad Sci U S A.* **2016,** *113,* E6831-E6839.
17. Libby, A. R. G.; Joy, D. A.; Elder, N. H.; Bulger, E. A.; Krakora, M. Z.; Gaylord, E. A.; Mendoza-Camacho, F.; Butts, J. C.; McDevitt, T. C. Axial elongation of caudalized human organoids mimics aspects of neural tube development. *Development.* **2021,** *148,* dev198275.
18. Ogura, T.; Sakaguchi, H.; Miyamoto, S.; Takahashi, J. Three-dimensional induction of dorsal, intermediate and ventral spinal cord tissues from human pluripotent stem cells. *Development.* **2018,** *145,* dev162214.
19. Duval, N.; Vaslin, C.; Barata, T. C.; Frarma, Y.; Contremoulins, V.; Baudin, X.; Nedelec, S.; Ribes, V. C. BMP4 patterns Smad activity and generates stereotyped cell fate organization in spinal organoids. *Development.* **2019,** *146,* dev175430.
20. Henrique, D.; Abranches, E.; Verrier, L.; Storey, K. G. Neuromesodermal progenitors and the making of the spinal cord. *Development.* **2015,** *142,* 2864-2875.
21. Whye, D.; Wood, D.; Kim, K. H.; Chen, C.; Makhortova, N.; Sahin, M.; Buttermore, E. D. Dynamic 3D combinatorial generation of hPSC-derived neuromesodermal organoids with diverse regional and cellular identities. *Curr Protoc.* **2022,** *2,* e568.
22. Gribaudo, S.; Robert, R.; van Sambeek, B.; Mirdass, C.; Lyubimova, A.; Bouhali, K.; Ferent, J.; Morin, X.; van Oudenaarden, A.; Nedelec, S. Self-organizing models of human trunk organogenesis recapitulate spinal cord and spine co-morphogenesis. *Nat Biotechnol.* **2024,** *42,* 1243-1253.
23. Yadav, A.; Matson, K. J. E.; Li, L.; Hua, I.; Petrescu, J.; Kang, K.; Alkaslasi, M. R.; Lee, D. I.; Hasan, S.; Galuta, A.; Dedek, A.; Ameri, S.; Parnell, J.; Alshardan, M. M.; Qumqumji, F. A.; Alhamad, S. M.; Wang, A. P.; Poulen, G.; Lonjon, N.; Vachiery-Lahaye, F.; Gaur, P.; Nalls, M. A.; Qi, Y. A.; Maric, D.; Ward, M. E.; Hildebrand, M. E.; Mery, P. F.; Bourinet, E.; Bauchet, L.; Tsai, E. C.; Phatnani, H.; Le Pichon, C. E.; Menon, V.; Levine, A. J. A cellular taxonomy of the adult human spinal cord. *Neuron.* **2023,** *111,* 328-344.e7.
24. Borromeo, M. D.; Meredith, D. M.; Castro, D. S.; Chang, J. C.; Tung, K. C.; Guillemot, F.; Johnson, J. E. A transcription factor network specifying inhibitory versus excitatory neurons in the dorsal spinal cord. *Development.* **2014,** *141,* 2803-2812.
25. Motojima, Y.; Ueta, Y.; Sakai, A. Analysis of the proportion and neuronal activity of excitatory and inhibitory neurons in the rat dorsal spinal cord after peripheral nerve injury. *Neurosci Lett.* **2021,** *749,* 135707.
26. Bertels, H.; Vicente-Ortiz, G.; El Kanbi, K.; Takeoka, A. Neurotransmitter phenotype switching by spinal excitatory interneurons regulates locomotor recovery after spinal cord injury. *Nat Neurosci.* **2022,** *25,* 617-629.
27. Miyazaki, Y.; Adachi, T.; Utsumi, J.; Shichino, T.; Segawa, H. Xenon

has greater inhibitory effects on spinal dorsal horn neurons than nitrous oxide in spinal cord transected cats. *Anesth Analg.* **1999,** *88,* 893-897.

28. Liu, M. Z.; Chen, X. J.; Liang, T. Y.; Li, Q.; Wang, M.; Zhang, X. Y.; Li, Y. Z.; Sun, Q.; Sun, Y. G. Synaptic control of spinal GRPR(+) neurons by local and long-range inhibitory inputs. *Proc Natl Acad Sci U S A.* **2019,** *116,* 27011-27017.
29. Serafin, E. K.; Burns, R.; Yoo, J.; Baccei, M. L. Gucy2d selectively marks inhibitory dynorphin neurons in the spinal dorsal horn but is dispensable for pain and itch sensitivity. *Pain Rep.* **2021,** *6,* e947.
30. Powis, R. A.; Gillingwater, T. H. Selective loss of alpha motor neurons with sparing of gamma motor neurons and spinal cord cholinergic neurons in a mouse model of spinal muscular atrophy. *J Anat.* **2016,** *228,* 443-451.
31. Kirby, A. J.; Palmer, T.; Mead, R. J.; Ichiyama, R. M.; Chakrabarty, S. Caudal-rostral progression of alpha motoneuron degeneration in the SOD1(G93A) mouse model of amyotrophic lateral sclerosis. *Antioxidants (Basel).* **2022,** *11,* 983.
32. Prochazka, A.; Mushahwar, V.; Yakovenko, S. Activation and coordination of spinal motoneuron pools after spinal cord injury. *Prog Brain Res.* **2002,** *137,* 109-124.
33. Gould, T. W.; Oppenheim, R. W. The function of neurotrophic factor receptors expressed by the developing adductor motor pool in vivo. *J Neurosci.* **2004,** *24,* 4668-4682.
34. Yokoyama, H.; Ogawa, T.; Shinya, M.; Kawashima, N.; Nakazawa, K. Speed dependency in α-motoneuron activity and locomotor modules in human locomotion: indirect evidence for phylogenetically conserved spinal circuits. *Proc Biol Sci.* **2017,** *284,* 20170290.
35. Panayiotou, E.; Panayi, E.; Lapathitis, G.; Francius, C.; Clotman, F.; Kessaris, N.; Richardson, W. D.; Malas, S. Pax6 is expressed in subsets of V0 and V2 interneurons in the ventral spinal cord in mice. *Gene Expr Patterns.* **2013,** *13,* 328-334.
36. Osseward, P. J., 2nd; Pfaff, S. L. Cell type and circuit modules in the spinal cord. *Curr Opin Neurobiol.* **2019,** *56,* 175-184.
37. Gray de Cristoforis, A.; Ferrari, F.; Clotman, F.; Vogel, T. Differentiation and localization of interneurons in the developing spinal cord depends on DOT1L expression. *Mol Brain.* **2020,** *13,* 85.
38. Cheng, J.; Guan, N. N. A fresh look at propriospinal interneurons plasticity and intraspinal circuits remodeling after spinal cord injury. *IBRO Neurosci Rep.* **2023,** *14,* 441-446.
39. Seo, W. M.; Yoon, J.; Lee, J. H.; Lee, Y.; Lee, H.; Geum, D.; Sun, W.; Song, M. R. Modeling axonal regeneration by changing cytoskeletal dynamics in stem cell-derived motor nerve organoids. *Sci Rep.* **2022,** *12,* 2082.
40. James, O. G.; Selvaraj, B. T.; Magnani, D.; Burr, K.; Connick, P.; Barton, S. K.; Vasistha, N. A.; Hampton, D. W.; Story, D.; Smigiel, R.; Ploski, R.; Brophy, P. J.; Ffrench-Constant, C.; Lyons, D. A.; Chandran, S. iPSC-derived myelinoids to study myelin biology of humans. *Dev Cell.* **2021,** *56,* 1346-1358.e6.
41. Muckom, R. J.; Sampayo, R. G.; Johnson, H. J.; Schaffer, D. V. Advanced materials to enhance central nervous system tissue modeling and cell therapy. *Adv Funct Mater.* **2020,** *30,* 2002931.
42. Shin, A.; Ryu, J. R.; Kim, B. G.; Sun, W. Establishment and validation of a model for fetal neural ischemia using necrotic core-free human spinal cord organoids. *Stem Cells Transl Med.* **2024,** *13,* 268-277.
43. Lee, J. H.; Shaker, M. R.; Park, S. H.; Sun, W. Transcriptional signature of valproic acid-induced neural tube defects in human spinal cord organoids. *Int J Stem Cells.* **2023,** *16,* 385-393.
44. Zou, H.; Wang, J. Y.; Ma, G. M.; Xu, M. M.; Luo, F.; Zhang, L.; Wang, W. Y. The function of FUS in neurodevelopment revealed by the brain and spinal cord organoids. *Mol Cell Neurosci.* **2022,** *123,* 103771.
45. Lee, J. H.; Shin, H.; Shaker, M. R.; Kim, H. J.; Park, S. H.; Kim, J. H.; Lee, N.; Kang, M.; Cho, S.; Kwak, T. H.; Kim, J. W.; Song, M. R.; Kwon, S. H.; Han, D. W.; Lee, S.; Choi, S. Y.; Rhyu, I. J.; Kim, H.; Geum, D.; Cho, I. J.; Sun, W. Production of human spinal-cord organoids recapitulating neural-tube morphogenesis. *Nat Biomed Eng.* **2022,** *6,* 435-448.
46. Winanto; Khong, Z. J.; Soh, B. S.; Fan, Y.; Ng, S. Y. Organoid cultures of MELAS neural cells reveal hyperactive Notch signaling that impacts neurodevelopment. *Cell Death Dis.* **2020,** *11,* 182.
47. Gao, C.; Shi, Q.; Pan, X.; Chen, J.; Zhang, Y.; Lang, J.; Wen, S.; Liu, X.; Cheng, T. L.; Lei, K. Neuromuscular organoids model spinal neuromuscular pathologies in C9orf72 amyotrophic lateral sclerosis. *Cell Rep.* **2024,** *43,* 113892.
48. Aguglia, G.; Coyne, C. B.; Dermody, T. S.; Williams, J. V.; Freeman, M. C. Contemporary enterovirus-D68 isolates infect human spinal cord organoids. *mBio.* **2023,** *14,* e0105823.
49. Grass, T.; Dokuzluoglu, Z.; Buchner, F.; Rosignol, I.; Thomas, J.; Caldarelli, A.; Dalinskaya, A.; Becker, J.; Rost, F.; Marass, M.; Wirth, B.; Beyer, M.; Bonaguro, L.; Rodriguez-Muela, N. Isogenic patient-derived organoids reveal early neurodevelopmental defects in spinal muscular atrophy initiation. *Cell Rep Med.* **2024,** *5,* 101659.
50. Ao, Z.; Cai, H.; Wu, Z.; Krzesniak, J.; Tian, C.; Lai, Y. Y.; Mackie, K.; Guo, F. Human spinal organoid-on-a-chip to model nociceptive circuitry for pain therapeutics discovery. *Anal Chem.* **2022,** *94,* 1365-1372.
51. Stiefel, D.; Shibata, T.; Meuli, M.; Duffy, P. G.; Copp, A. J. Tethering of the spinal cord in mouse fetuses and neonates with spina bifida. *J Neurosurg.* **2003,** *99,* 206-213.
52. Lundin, B. F.; Knight, G. T.; Fedorchak, N. J.; Krucki, K.; Iyer, N.; Maher, J. E.; Izban, N. R.; Roberts, A.; Cicero, M. R.; Robinson, J. F.; Iskandar, B. J.; Willett, R.; Ashton, R. S. RosetteArray(®) platform for quantitative high-throughput screening of human neurodevelopmental risk. *bioRxiv.* **2024.** doi: 10.1101/2024.04.01.587605.
53. Strong, M. J.; Strong, W. L.; Jaffe, H.; Traggert, B.; Sopper, M. M.; Pant, H. C. Phosphorylation state of the native high-molecular-weight neurofilament subunit protein from cervical spinal cord in sporadic amyotrophic lateral sclerosis. *J Neurochem.* **2001,** *76,* 1315-1325.
54. Schirmer, L.; Albert, M.; Buss, A.; Schulz-Schaeffer, W. J.; Antel, J. P.; Brück, W.; Stadelmann, C. Substantial early, but nonprogressive neuronal loss in multiple sclerosis (MS) spinal cord. *Ann Neurol.* **2009,** *66,* 698-704.
55. Liu, D.; Liu, C.; Li, J.; Azadzoi, K.; Yang, Y.; Fei, Z.; Dou, K.; Kowall, N. W.; Choi, H. P.; Vieira, F.; Yang, J. H. Proteomic analysis reveals differentially regulated protein acetylation in human amyotrophic lateral sclerosis spinal cord. *PLoS One.* **2013,** *8,* e80779.
56. Valbuena, G. N.; Cantoni, L.; Tortarolo, M.; Bendotti, C.; Keun, H. C. Spinal cord metabolic signatures in models of fast- and slow-progressing SOD1(G93A) amyotrophic lateral sclerosis. *Front Neurosci.* **2019,** *13,* 1276.
57. Zhao, A.; Pan, Y.; Cai, S. Patient-specific cells for modeling and decoding amyotrophic lateral sclerosis: advances and challenges. *Stem Cell Rev Rep.* **2020,** *16,* 482-502.
58. Chooi, W. H.; Ng, C. Y.; Ow, V.; Harley, J.; Ng, W.; Hor, J. H.; Low, K. E.; Malleret, B.; Xue, K.; Ng, S. Y. Defined alginate hydrogels support spinal cord organoid derivation, maturation, and modeling of spinal

cord diseases. *Adv Healthc Mater.* **2023,** *12,* e2202342.
59. Guo, R.; Chen, Y.; Zhang, J.; Zhou, Z.; Feng, B.; Du, X.; Liu, X.; Ma, J.; Cui, H. Neural Differentiation and spinal cord organoid generation from induced pluripotent stem cells (iPSCs) for ALS modelling and inflammatory screening. *Mol Neurobiol.* **2024,** *61,* 4732-4749.
60. Sirtori, R.; M, J. G.; E, M. P.; Collins, A.; Donatelli, L.; Fallini, C. LINC complex alterations are a key feature of sporadic and familial ALS/FTD. *Acta Neuropathol Commun.* **2024,** *12,* 69.
61. Han, Y.; Yang, L.; Lacko, L. A.; Chen, S. Human organoid models to study SARS-CoV-2 infection. *Nat Methods.* **2022,** *19,* 418-428.
62. Willner, M. J.; Xiao, Y.; Kim, H. S.; Chen, X.; Xu, B.; Leong, K. W. Modeling SARS-CoV-2 infection in individuals with opioid use disorder with brain organoids. *J Tissue Eng.* **2021,** *12,* 2041731420985299.
63. Hu, X.; Xu, W.; Ren, Y.; Wang, Z.; He, X.; Huang, R.; Ma, B.; Zhao, J.; Zhu, R.; Cheng, L. Spinal cord injury: molecular mechanisms and therapeutic interventions. *Signal Transduct Target Ther.* **2023,** *8,* 245.
64. Xu, J.; Fang, S.; Deng, S.; Li, H.; Lin, X.; Huang, Y.; Chung, S.; Shu, Y.; Shao, Z. Generation of neural organoids for spinal-cord regeneration via the direct reprogramming of human astrocytes. *Nat Biomed Eng.* **2023,** *7,* 253-269.
65. Li, X.; Fu, J.; Guan, M.; Shi, H.; Pan, W.; Lou, X. Biochanin A attenuates spinal cord injury in rats during early stages by inhibiting oxidative stress and inflammasome activation. *Neural Regen Res.* **2024,** *19,* 2050-2056.
66. Anjum, A.; Yazid, M. D.; Fauzi Daud, M.; Idris, J.; Ng, A. M. H.; Selvi Naicker, A.; Ismail, O. H. R.; Athi Kumar, R. K.; Lokanathan, Y. Spinal cord injury: pathophysiology, multimolecular interactions, and underlying recovery mechanisms. *Int J Mol Sci.* **2020,** *21,* 7533.
67. Lai, J. D.; Berlind, J. E.; Fricklas, G.; Lie, C.; Urenda, J. P.; Lam, K.; Sta Maria, N.; Jacobs, R.; Yu, V.; Zhao, Z.; Ichida, J. K. KCNJ2 inhibition mitigates mechanical injury in a human brain organoid model of traumatic brain injury. *Cell Stem Cell.* **2024,** *31,* 519-536.e8.
68. Deng, Y. F.; Xiang, P.; Du, J. Y.; Liang, J. F.; Li, X. Intrathecal liproxstatin-1 delivery inhibits ferroptosis and attenuates mechanical and thermal hypersensitivities in rats with complete Freund's adjuvant-induced inflammatory pain. *Neural Regen Res.* **2023,** *18,* 456-462.
69. Li, D. Y.; Gao, S. J.; Sun, J.; Zhang, L. Q.; Wu, J. Y.; Song, F. H.; Liu, D. Q.; Zhou, Y. Q.; Mei, W. Targeting the nitric oxide/cGMP signaling pathway to treat chronic pain. *Neural Regen Res.* **2023,** *18,* 996-1003.
70. Xiao, D.; Deng, Q.; Guo, Y.; Huang, X.; Zou, M.; Zhong, J.; Rao, P.; Xu, Z.; Liu, Y.; Hu, Y.; Shen, Y.; Jin, K.; Xiang, M. Generation of self-organized sensory ganglion organoids and retinal ganglion cells from fibroblasts. *Sci Adv.* **2020,** *6,* eaaz5858.
71. Yang, J.; Yan, Y.; Yin, X.; Liu, X.; Reshetov, I. V.; Karalkin, P. A.; Li, Q.; Huang, R. L. Bioengineering and vascularization strategies for islet organoids: advancing toward diabetes therapy. *Metabolism.* **2024,** *152,* 155786.
72. Wang, M.; Gage, F. H.; Schafer, S. T. Transplantation strategies to enhance maturity and cellular complexity in brain organoids. *Biol Psychiatry.* **2023,** *93,* 616-621.
73. Kofman, S.; Mohan, N.; Sun, X.; Ibric, L.; Piermarini, E.; Qiang, L. Human mini brains and spinal cords in a dish: Modeling strategies, current challenges, and prospective advances. *J Tissue Eng.* **2022,** *13,* 20417314221113391.
74. Xue, W.; Li, B.; Liu, H.; Xiao, Y.; Li, B.; Ren, L.; Li, H.; Shao, Z. Generation of dorsoventral human spinal cord organoids via functionalizing composite scaffold for drug testing. *iScience.* **2023,** *26,* 105898.
75. Lancaster, M. A.; Corsini, N. S.; Wolfinger, S.; Gustafson, E. H.; Phillips, A. W.; Burkard, T. R.; Otani, T.; Livesey, F. J.; Knoblich, J. A. Guided self-organization and cortical plate formation in human brain organoids. *Nat Biotechnol.* **2017,** *35,* 659-666.
76. Li, W.; Zhang, G.; Guan, T.; Zhang, X.; Khosrozadeh, A.; Xing, M.; Kong, J. Manipulable permeability of nanogel encapsulation on cells exerts protective effect against TNF-α-induced apoptosis. *ACS Biomater Sci Eng.* **2018,** *4,* 2825-2835.
77. Wu, W.; Liu, Y.; Liu, R.; Wang, Y.; Zhao, Y.; Li, H.; Lu, B.; Ju, C.; Gao, X.; Xu, H.; Cao, Y.; Cheng, S.; Wang, Z.; Jia, S.; Hu, C.; Zhu, L.; Hao, D. Decellularized brain extracellular matrix hydrogel aids the formation of human spinal-cord organoids recapitulating the complex three-dimensional organization. *ACS Biomater Sci Eng.* **2024,** *10,* 3203-3217.
78. Sun, Z.; Chen, Z.; Yin, M.; Wu, X.; Guo, B.; Cheng, X.; Quan, R.; Sun, Y.; Zhang, Q.; Fan, Y.; Jin, C.; Yin, Y.; Hou, X.; Liu, W.; Shu, M.; Xue, X.; Shi, Y.; Chen, B.; Xiao, Z.; Dai, J.; Zhao, Y. Harnessing developmental dynamics of spinal cord extracellular matrix improves regenerative potential of spinal cord organoids. *Cell Stem Cell.* **2024,** *31,* 772-787.e11.
79. Wang, Z.; Liu, R.; Liu, Y.; Zhao, Y.; Wang, Y.; Lu, B.; Li, H.; Ju, C.; Wu, W.; Gao, X.; Xu, H.; Cheng, S.; Cao, Y.; Jia, S.; Hu, C.; Zhu, L.; Hao, D. Human placenta decellularized extracellular matrix hydrogel promotes the generation of human spinal cord organoids with dorsoventral organization from human induced pluripotent stem cells. *ACS Biomater Sci Eng.* **2024,** *10,* 3218-3231.
80. Han, Y.; King, M.; Tikhomirov, E.; Barasa, P.; Souza, C. D. S.; Lindh, J.; Baltriukiene, D.; Ferraiuolo, L.; Azzouz, M.; Gullo, M. R.; Kozlova, E. N. Towards 3D bioprinted spinal cord organoids. *Int J Mol Sci.* **2022,** *23,* 5788.
81. Lai, B. Q.; Feng, B.; Che, M. T.; Wang, L. J.; Cai, S.; Huang, M. Y.; Gu, H. Y.; Jiang, B.; Ling, E. A.; Li, M.; Zeng, X.; Zeng, Y. S. A modular assembly of spinal cord-like tissue allows targeted tissue repair in the transected spinal cord. *Adv Sci (Weinh).* **2018,** *5,* 1800261.
82. Lai, B. Q.; Wu, R. J.; Han, W. T.; Bai, Y. R.; Liu, J. L.; Yu, H. Y.; Yang, S. B.; Wang, L. J.; Ren, J. L.; Ding, Y.; Li, G.; Zeng, X.; Ma, Y. H.; Quan, Q.; Xing, L. Y.; Jiang, B.; Wang, Y. Q.; Zhang, L.; Chen, Z. H.; Zhang, H. B.; Chen, Y. F.; Zheng, Q. J.; Zeng, Y. S. Tail nerve electrical stimulation promoted the efficiency of transplanted spinal cord-like tissue as a neuronal relay to repair the motor function of rats with transected spinal cord injury. *Biomaterials.* **2023,** *297,* 122103.
83. Ji, Y.; Li, Y. M.; Seo, J. G.; Jang, T. S.; Knowles, J. C.; Song, S. H.; Lee, J. H. Biological potential of polyethylene glycol (PEG)-functionalized graphene quantum dots in in vitro neural stem/progenitor cells. *Nanomaterials (Basel).* **2021,** *11,* 1446.
84. Ito, Y.; Oyane, A.; Yasunaga, M.; Hirata, K.; Hirose, M.; Tsurushima, H.; Ito, Y.; Matsumaru, Y.; Ishikawa, E. Induction of angiogenesis and neural progenitor cells by basic fibroblast growth factor-releasing polyglycolic acid sheet following focal cerebral infarction in mice. *J Biomed Mater Res A.* **2022,** *110,* 1964-1975.
85. Layrolle, P.; Payoux, P.; Chavanas, S. Message in a scaffold: natural biomaterials for three-dimensional (3D) bioprinting of human brain organoids. *Biomolecules.* **2022,** *13,* 25.
86. Marchini, A.; Gelain, F. Synthetic scaffolds for 3D cell cultures and organoids: applications in regenerative medicine. *Crit Rev Biotechnol.* **2022,** *42,* 468-486.
87. Kim, S.; Min, S.; Choi, Y. S.; Jo, S. H.; Jung, J. H.; Han, K.; Kim, J.; An, S.; Ji, Y. W.; Kim, Y. G.; Cho, S. W. Tissue extracellular matrix

hydrogels as alternatives to Matrigel for culturing gastrointestinal organoids. *Nat Commun.* **2022,** *13*, 1692.
88. Pham, M. T.; Pollock, K. M.; Rose, M. D.; Cary, W. A.; Stewart, H. R.; Zhou, P.; Nolta, J. A.; Waldau, B. Generation of human vascularized brain organoids. *Neuroreport.* **2018,** *29*, 588-593.
89. Kelava, I.; Lancaster, M. A. Dishing out mini-brains: current progress and future prospects in brain organoid research. *Dev Biol.* **2016,** *420*, 199-209.
90. Zhang, Z. N.; Freitas, B. C.; Qian, H.; Lux, J.; Acab, A.; Trujillo, C. A.; Herai, R. H.; Nguyen Huu, V. A.; Wen, J. H.; Joshi-Barr, S.; Karpiak, J. V.; Engler, A. J.; Fu, X. D.; Muotri, A. R.; Almutairi, A. Layered hydrogels accelerate iPSC-derived neuronal maturation and reveal migration defects caused by MeCP2 dysfunction. *Proc Natl Acad Sci U S A.* **2016,** *113*, 3185-3190.
91. Wei, Y. T.; He, Y.; Xu, C. L.; Wang, Y.; Liu, B. F.; Wang, X. M.; Sun, X. D.; Cui, F. Z.; Xu, Q. Y. Hyaluronic acid hydrogel modified with nogo-66 receptor antibody and poly-L-lysine to promote axon regrowth after spinal cord injury. *J Biomed Mater Res B Appl Biomater.* **2010,** *95*, 110-117.
92. Xiang, Y.; Tanaka, Y.; Patterson, B.; Kang, Y. J.; Govindaiah, G.; Roselaar, N.; Cakir, B.; Kim, K. Y.; Lombroso, A. P.; Hwang, S. M.; Zhong, M.; Stanley, E. G.; Elefanty, A. G.; Naegele, J. R.; Lee, S. H.; Weissman, S. M.; Park, I. H. Fusion of regionally specified hPSC-derived organoids models human brain development and interneuron migration. *Cell Stem Cell.* **2017,** *21*, 383-398.e7.
93. Hoang, P.; Ma, Z. Biomaterial-guided stem cell organoid engineering for modeling development and diseases. *Acta Biomater.* **2021,** *132*, 23-36.
94. He, F.; Tao, T.; Liu, H.; Wang, Y.; Cui, K.; Guo, Y.; Qin, J. Controllable fabrication of composite core-shell capsules at a macroscale as organoid biocarriers. *ACS Appl Bio Mater.* **2021,** *4*, 1584-1596.
95. Wertheim, L.; Edri, R.; Goldshmit, Y.; Kagan, T.; Noor, N.; Ruban, A.; Shapira, A.; Gat-Viks, I.; Assaf, Y.; Dvir, T. Regenerating the injured spinal cord at the chronic phase by engineered iPSCs-derived 3D neuronal networks. *Adv Sci (Weinh).* **2022,** *9*, e2105694.
96. Feng, B.; Yang, H.; Zhu, M.; Li, J.; Chang, H. M.; Leung, P. C. K.; Guo, J.; Zhang, Y. Collagen-based biomaterials in organoid technology for reproductive medicine: composition, characteristics, and applications. *Collagen Leather.* **2023,** *5*, 35.
97. Chen, Z. X.; Zha, X. J.; Xia, Y. K.; Ling, T. X.; Xiong, J.; Huang, J. G. 3D foaming printing biomimetic hierarchically macro-micronanoporous hydrogels for enhancing cell growth and proliferation. *ACS Appl Mater Interfaces.* **2024,** *16*, 10813-10821.
98. Chen, X.; Wang, Y.; Zhou, G.; Hu, X.; Han, S.; Gao, J. The combination of nanoscaffolds and stem cell transplantation: Paving a promising road for spinal cord injury regeneration. *Biomed Pharmacother.* **2021,** *143*, 112233.
99. Mansour, A. A.; Gonçalves, J. T.; Bloyd, C. W.; Li, H.; Fernandes, S.; Quang, D.; Johnston, S.; Parylak, S. L.; Jin, X.; Gage, F. H. An in vivo model of functional and vascularized human brain organoids. *Nat Biotechnol.* **2018,** *36*, 432-441.
100. Lim, S.; Jung, G. A.; Muckom, R. J.; Glover, D. J.; Clark, D. S. Engineering bioorthogonal protein-polymer hybrid hydrogel as a functional protein immobilization platform. *Chem Commun (Camb).* **2019,** *55*, 806-809.
101. Mirabella, T.; MacArthur, J. W.; Cheng, D.; Ozaki, C. K.; Woo, Y. J.; Yang, M.; Chen, C. S. 3D-printed vascular networks direct therapeutic angiogenesis in ischaemia. *Nat Biomed Eng.* **2017,** *1*, 0083.
102. Urciuolo, A.; Giobbe, G. G.; Dong, Y.; Michielin, F.; Brandolino, L.; Magnussen, M.; Gagliano, O.; Selmin, G.; Scattolini, V.; Raffa, P.; Caccin, P.; Shibuya, S.; Scaglioni, D.; Wang, X.; Qu, J.; Nikolic, M.; Montagner, M.; Galea, G. L.; Clevers, H.; Giomo, M.; De Coppi, P.; Elvassore, N. Hydrogel-in-hydrogel live bioprinting for guidance and control of organoids and organotypic cultures. *Nat Commun.* **2023,** *14*, 3128.
103. Knight, G. T.; Lundin, B. F.; Iyer, N.; Ashton, L. M.; Sethares, W. A.; Willett, R. M.; Ashton, R. S. Engineering induction of singular neural rosette emergence within hPSC-derived tissues. *Elife.* **2018,** *7*, e37549.
104. Seo, K.; Cho, S.; Shin, H.; Shin, A.; Lee, J. H.; Kim, J. H.; Lee, B.; Jang, H.; Kim, Y.; Cho, H. M.; Park, Y.; Kim, H. Y.; Lee, T.; Park, W. Y.; Kim, Y. J.; Yang, E.; Geum, D.; Kim, H.; Cho, I. J.; Lee, S.; Ryu, J. R.; Sun, W. Symmetry breaking of human pluripotent stem cells (hPSCs) in micropattern generates a polarized spinal cord-like organoid (pSCO) with dorsoventral organization. *Adv Sci (Weinh).* **2023,** *10*, e2301787.
105. Anand, G. M.; Megale, H. C.; Murphy, S. H.; Weis, T.; Lin, Z.; He, Y.; Wang, X.; Liu, J.; Ramanathan, S. Controlling organoid symmetry breaking uncovers an excitable system underlying human axial elongation. *Cell.* **2023,** *186*, 497-512.e23.
106. Xue, X.; Kim, Y. S.; Ponce-Arias, A. I.; O'Laughlin, R.; Yan, R. Z.; Kobayashi, N.; Tshuva, R. Y.; Tsai, Y. H.; Sun, S.; Zheng, Y.; Liu, Y.; Wong, F. C. K.; Surani, A.; Spence, J. R.; Song, H.; Ming, G. L.; Reiner, O.; Fu, J. A patterned human neural tube model using microfluidic gradients. *Nature.* **2024,** *628*, 391-399.
107. Andersen, J.; Revah, O.; Miura, Y.; Thom, N.; Amin, N. D.; Kelley, K. W.; Singh, M.; Chen, X.; Thete, M. V.; Walczak, E. M.; Vogel, H.; Fan, H. C.; Paşca, S. P. Generation of functional human 3D cortico-motor assembloids. *Cell.* **2020,** *183*, 1913-1929.e26.
108. Faustino Martins, J. M.; Fischer, C.; Urzi, A.; Vidal, R.; Kunz, S.; Ruffault, P. L.; Kabuss, L.; Hube, I.; Gazzerro, E.; Birchmeier, C.; Spuler, S.; Sauer, S.; Gouti, M. Self-organizing 3D human trunk neuromuscular organoids. *Cell Stem Cell.* **2020,** *27*, 498.
109. Son, J.; Park, S. J.; Ha, T.; Lee, S. N.; Cho, H. Y.; Choi, J. W. Electrophysiological monitoring of neurochemical-based neural signal transmission in a human brain-spinal cord assembloid. *ACS Sens.* **2022,** *7*, 409-414.
110. Hong, S.; Lee, J.; Kim, Y.; Kim, E.; Shin, K. AAVS1-targeted, stable expression of ChR2 in human brain organoids for consistent optogenetic control. *Bioeng Transl Med.* **2024**, e10690.
111. Hor, J. H.; Soh, E. S.; Tan, L. Y.; Lim, V. J. W.; Santosa, M. M.; Winanto; Ho, B. X.; Fan, Y.; Soh, B. S.; Ng, S. Y. Cell cycle inhibitors protect motor neurons in an organoid model of Spinal Muscular Atrophy. *Cell Death Dis.* **2018,** *9*, 1100.
112. Li, M.; Gao, L.; Zhao, L.; Zou, T.; Xu, H. Toward the next generation of vascularized human neural organoids. *Med Res Rev.* **2023,** *43*, 31-54.
113. Tang, X. Y.; Wu, S.; Wang, D.; Chu, C.; Hong, Y.; Tao, M.; Hu, H.; Xu, M.; Guo, X.; Liu, Y. Human organoids in basic research and clinical applications. *Signal Transduct Target Ther.* **2022,** *7*, 168.
114. O'Hara-Wright, M.; Mobini, S.; Gonzalez-Cordero, A. Bioelectric potential in next-generation organoids: electrical stimulation to enhance 3D structures of the central nervous system. *Front Cell Dev Biol.* **2022,** *10*, 901652.

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