



Converging neural-centric and mechano-regulation in organoid modeling for system biology and medicine

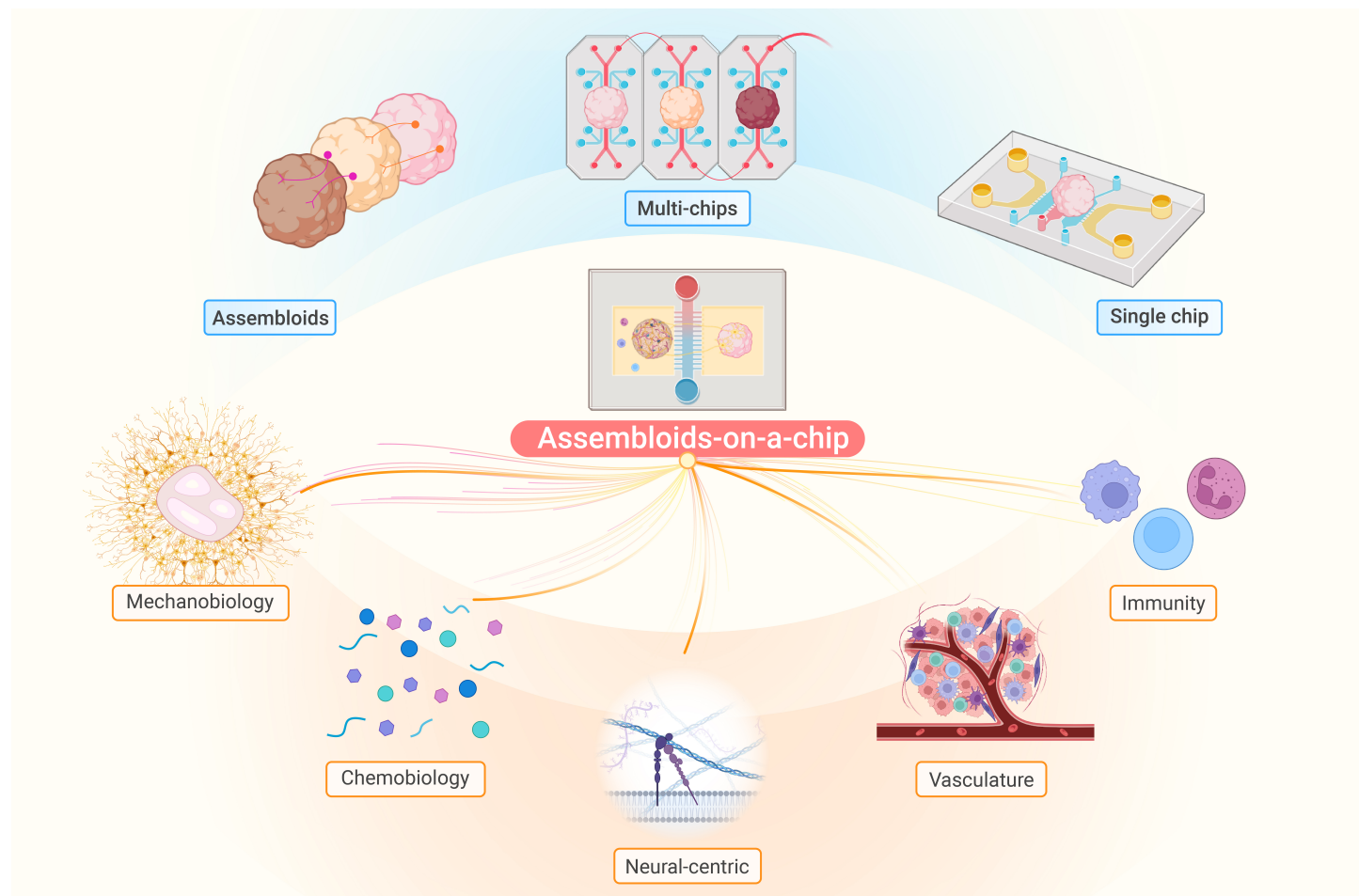
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Received: November 29, 2023; Accepted: April 12, 2024; Published Online: June 14, 2024; <https://doi.org/10.59717/j.xinn-med.2024.100076>

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GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Using organoids derivatives for modeling complex biological systems and improving precision medicine.
- By integrating the neuroregulatory and mechanobiological factors can simulate multi-organ interactions.
- Organoids and their derivatives represent a significant biomimetic, robust in-vitro models.

Converging neural-centric and mechano-regulation in organoid modeling for system biology and medicine

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Citation: Tang J., Wang Z., Khutsishvili D., et al., (2024). Converging neural-centric and mechano-regulation in organoid modeling for system biology and medicine. *The Innovation Medicine* 2(3): 100076.

The understanding of complex biological systems and the development of effective precision medicine strategies necessitate controllable and tractable experimental models. The human body is composed of systemic and systematic interactions at multiple levels such as occurs between cells, tissues, and organs. Hence, how to recapitulate the system complexity of the human body has become an inevitable problem. This review emphasizes the need to understand complex interactions between organs by exploring the potential use of organoids and their derivatives. We focus on the nervous system and its pivotal roles in the regulation of peripheral organs, and meanwhile, highlight the importance of often overlooked mechanobiological factors. The nervous system controls many neuromodulation processes and is capable of transmitting biological information through electrophysiology. In addition, mechano-regulation operates at the cellular and microenvironment levels, functioning through system-level regulation. It can influence neural tissue or collaborate with nerves to direct skin and visceral tissue responses and their immunity. To achieve *in situ* probing and manipulation of such processes, we recommend the use of organoid assembloids that directly fusion individual organoids to create interactive structures of neural-centric complexes and mechano-regulation conditions, or organoids-on-a-chip that relies on microfluidic chips as tailorable bioreactors to form multi-organ associations and simulate and incorporate mechanobiological and neurological regulations. Based on mechano-regulatory influence at the cellular and microenvironment levels, we may develop more systematic, biomimetic, and robust *in-vitro* models. These models not only approach genuine physiology and pathology in humans without sacrificing real-time observation capabilities but present minimal ethical concerns and offer substantial potential for industrial scalability.

INTRODUCTION

Organoids, as an innovative technique for modeling human organ development and disease progression, have seen remarkable advancement over the past two decades. However, the human body, a complex composite of various systems and individual organs, encompasses intricate developmental and regulatory processes that respond to an array of environmental conditions and diseases, such as metabolic and immune responses. While much of past research has centered on the development of specific organ regions, offering invaluable insights into the unique aspects of individual organ development. Such a narrow lens often results in a superficial grasp of essential developmental processes and the pathogenesis of multiple diseases. Studies on organoid modeling have traditionally focused on understanding and differentiating the roles of individual elements. Further development demands and directs the application of organoid technology to system modeling. The core issue in designing related models is the trade-off between the complexity of biological systems and the degree of engineering simplification. This trade-off determines how to integrate and extend modularity with reference to the real system while meeting the high-throughput needs of manufacturing and application in engineering means (Figure 1). To address this, the field has seen the emergence of tools such as organoids-on-a-chip and assembloids. These methodologies, derived from the versatile nature of organoids, empha-

size their remarkable ability for adaptability, integration, and interactivity, underscoring their potential in bridging the gap in understanding.

In this review, we delve into the intricate realm of system interactions. Incorporating the nervous system and mechanobiological insights, we elucidate how system biology operates not only at cellular and tissue levels but also extends to complex organ interactions, each with its unique innervation and mechano-regulation. By harnessing the classic neuromodulation intervention process, particularly emphasizing the brain-gut axis, we highlight the necessity of examining human physiological functions and disease pathology through the integrated perspective of multi-organ interactions. Concurrently, we harness the three-dimensional (3D) spatial features presented by organoid models, along with established and reliable bio-mimicry technologies such as microfluidics, to investigate the influence of biochemical and mechanobiological factors on system interactions. This approach enables us to investigate the role of mechanobiological factors in system interactions, thereby enhancing our overall understanding under the joint guidance of chemical and physical principles.

ORGANOIDS AS MODELING FOR HUMAN SYSTEM ELEMENTS AND INTERACTIONS

The human body possesses a multi-level and systemic nature, which puts forward three requirements for the selection of experimental models: modifiable constituent elements, manageable experimental settings, and observable experimental outcomes. In practice, traditional animal models often entail a complex biological system, which can result in a loss of control over the constitutive parameters. It is also difficult to detect the physiological changes in the animal body in real-time and intrinsic species-specific differences exist compared to the human phenotype. The advent of organoid technology, when synergized with microfluidic technology, meets the outlined requirements for studying human physiology, pathology, and responses to external factors, such as drug interventions, in the formats of organoids, organoid assembloids, and organoids-on-a-chip configurations. The purpose of organoid modeling is to utilize the self-organization and differentiation abilities of stem cells or primary tissue cells to replicate the developmental process and physiological response of human organs as closely as possible.^{1,2} Currently, numerous organoid protocols for organs such as the brain,^{3,4} lung,⁵ and kidney⁶ have successfully replicated the process of cell differentiation and tissue structure polarization observed in organ development and ultimately exhibited significant morphological and phenotypic overlap with human organs.^{7,8} This makes it possible to use organoids to study unknown processes involved in structural development,⁹ functional principles,¹⁰ pathology,¹¹ and drug responses.¹² (Figure 2, Organ)

The single organoid is the elemental basis

Brain. Brain organoids have undergone significant development since their inception.¹³⁻¹⁵ These advances have birthed unguided brain organoids¹⁶⁻¹⁸ that do not necessitate patterning factors and are mainly comprised of ectoderm-derived cells. Moreover, they may now also incorporate, or even generate, cells of mesodermal origin, such as microglia. Furthermore, guided brain organoids,¹⁹⁻²¹ which are induced by patterning factors to form specific brain regions, such as cortical organoids,^{19,22} provide a purer source of ectodermal

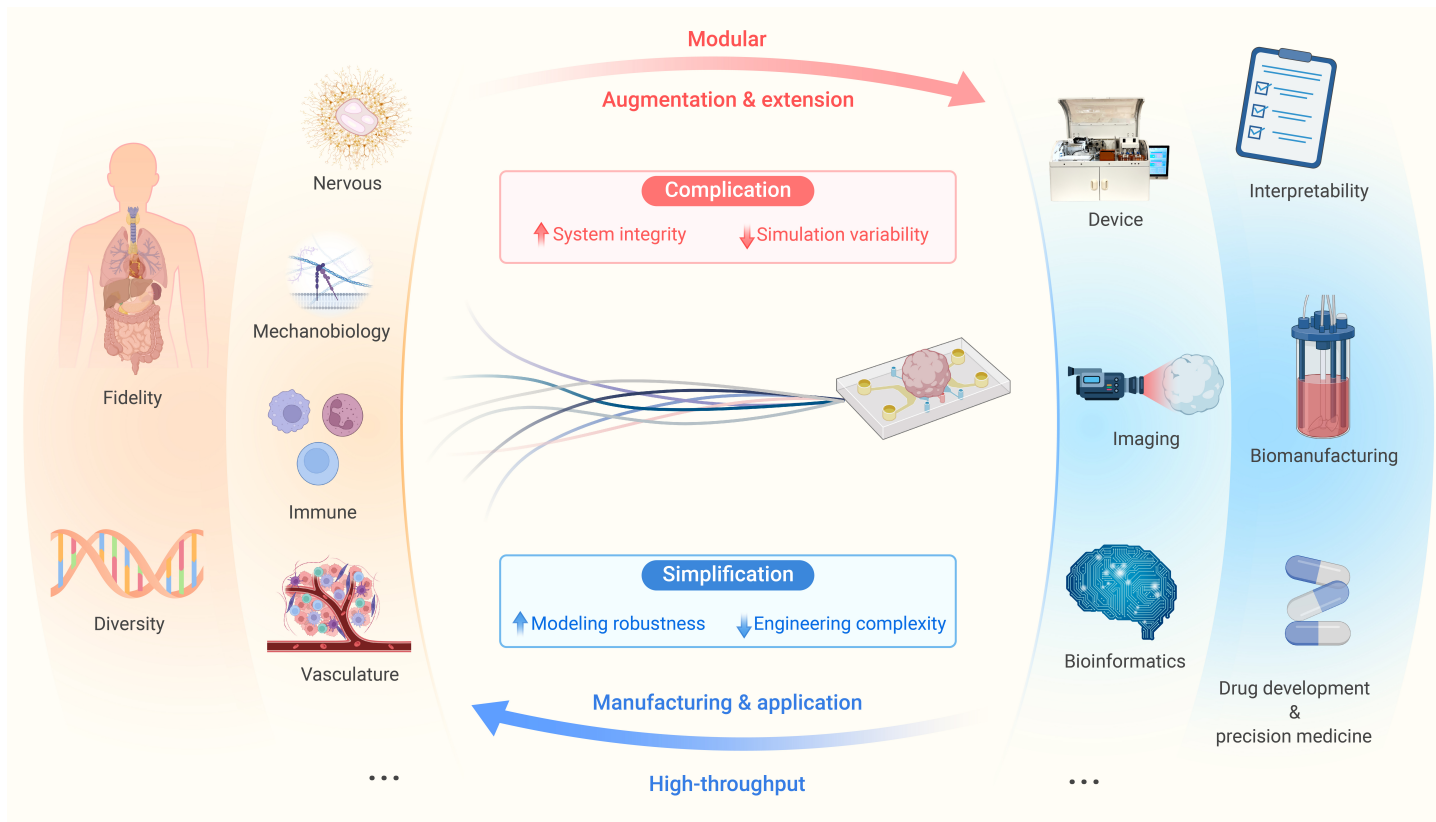


Figure 1. Complication of Biology and Simplification of Engineering The aim of designing a systematized organoid-on-a-chip is to enhance the complexity of biological systems while reducing simulation discrepancies. Simultaneously, it aims to enhance the robustness of models in engineering and reduce engineering complexity. This approach ensures that the integration and extension of modules in the organoid-on-a-chip are directed by the real biological system while meeting the high-throughput and high-reproducibility requirements for engineering, manufacturing, and applications. It enables the integration of engineering means, such as instrumentation, imaging, and information acquisition, into biological interpretability, manufacturing, and drug discovery and development. It meanwhile possesses wide-spectrum compatibility with diverse biological samples.

development with improved consistency than the unguided method. Through years of optimization, stable brain organoid culture protocols have been established on this basis.^{17,23,24} For investigating human brain development, studies have been conducted to analyze changes in cell components (such as cell polarity^{15,25,26} and differentiation),^{16,27} molecular pathways,^{28,29} structures (such as modeling and studying ventricular zone structure,²² folding patterns,⁹ and nerve tract projections),³⁰ and electrophysiological activity (such as oscillatory waves³¹ or the GABA polarity switch)³² during specific developmental stages. These studies have allowed researchers to observe the activity of neurons (such as inner-region self-organization²² and cross-region connections with migration)^{33,34} and have provided more valuable insights into human brain function compared to other non-human species.³⁵ In addition, related disease models, such as Alzheimer's disease^{36,37} and viral infection,¹⁹ especially disease models such as malformations of cortical development³⁸ have been established, providing new avenues for studying neuropathology, the neuroimmune system, and developing potential treatments.

The spinal cord, visceral organs, and skin. In recent years, the construction of spinal cord organoids has become feasible by applying a specific combination of molecular inhibitors and growth factors to direct the differentiation of embryonic bodies (EBs). Subsequently, the morphogenesis of the neural tube was replicated and examined using spinal cord organoids derived from pluripotent stem cells (PSCs).³⁹ The successfully generated spinal cord organoid can be utilized to model the diseases associated with neural-tube defects.³⁹ However, the rostral-caudal linear structure is still lacking in the current techniques to generate spinal cord organoids, which are hypothesized to construct spinal cord organoids through a liner arrangement.

The advances in organoid technology have also fostered the study of endogenous development processes, disease modeling, and translational application. At present, integumentary, intestinal, gastric, hepatic, pancreatic, renal, retinal, tonsillar, and prostatic organoids derived from pluripotent stem

cells and organ-restricted adult stem cells have been generated.^{40,41} For example, Lee et al. established a complex skin organoid with high cellular heterogeneity and a well-organized stratified epidermal layer.⁴² This strategy remarkably replicated the primary integumentary system structure with pigmented hair follicles, sebaceous glands, Merkel cells, and sensory neurons in stratified layers. Moreover, some organoids, such as cardiac organoids, are actively being transformed into clinical applications.⁴³ It should be noted that engineering techniques can improve the efficacy of organoid modeling by utilizing concentration gradients. For example, the extrusion-printed kidney organoid,⁴⁴ the cube method,⁴⁵ or microfluidics for creating concentration gradient, which shows parallels with organoids-on-chip discussed later.

Microenvironment is the bridge of intra-system interaction

The specificity of cell or organ function determines that a co-operative working system is required to realize complex somatic functions. At the subcellular level, this system comprises the nucleus, cell membrane, and organelles (Figure 2, Cell). At the tissue level, the system involves the coordination of cells, and the ECM, such as immune cells and stroma in the immune response (Figure 2, Microenvironment). Subsequently, at the level of tissue co-operation, these cells and the ECM collaborate to form complex organ structures (Figure 2, Tissue & Organ). Ultimately, this culminates in the intricate interactions between multiple organs across human organ systems (Figure 2, System).

The microenvironment encompasses various elements, including co-existing cells, complex chemical factors (such as ions, proteins, and lipids), and diverse physical factors (such as flow, space, and force). In contrast to the absence of an appropriate microenvironment in two-dimensional (2D) cultures and the uncontrolled interplay observed in animal models, organoids possess 3D characteristics that allow for the restoration of regional interactions among cells, and the composition parameters of the microenvironment can be precisely controlled, enabling quantitative research recapitulating

Mapping of Human System and Organoid

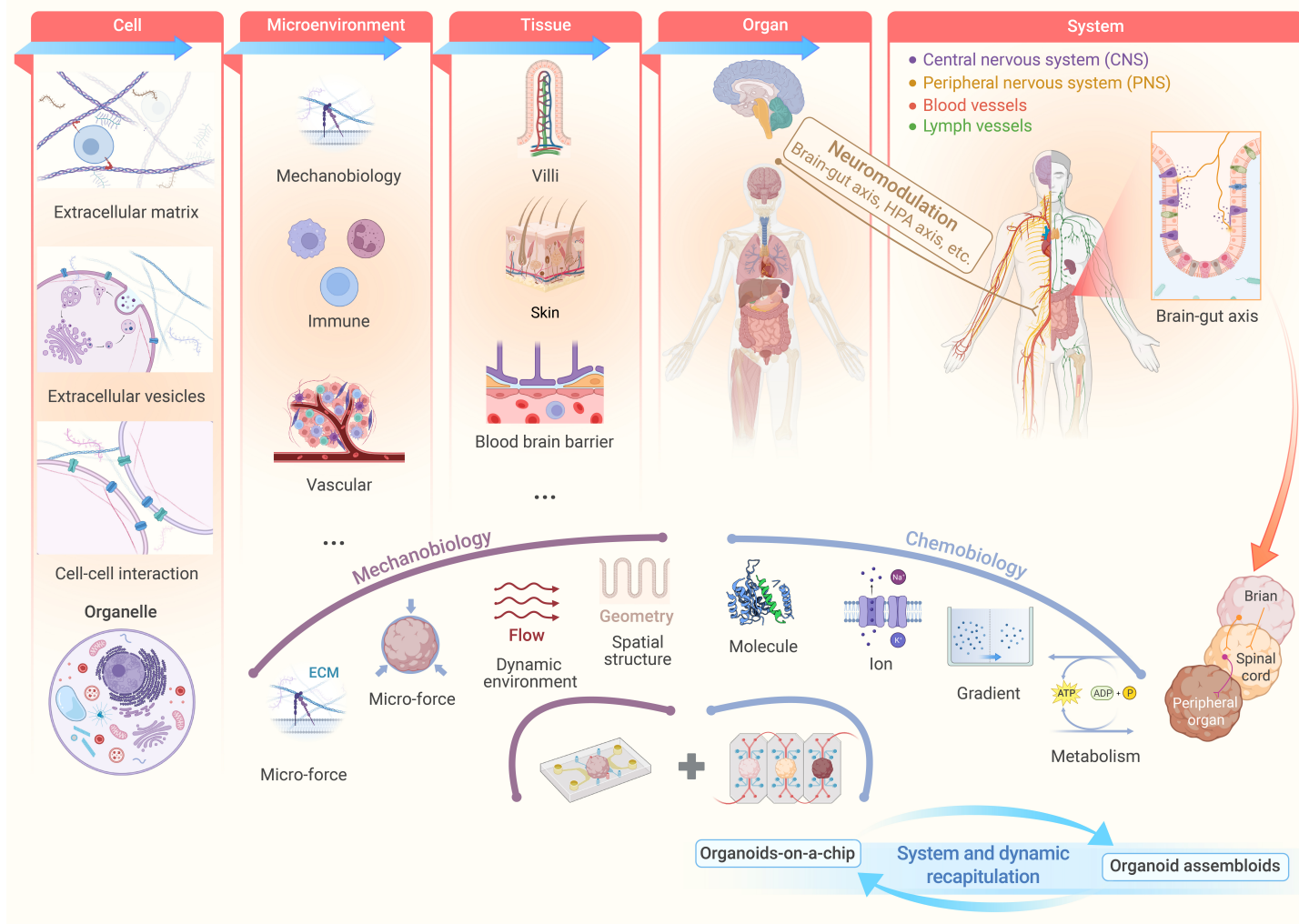


Figure 2. Mapping of human system and organoid The human body comprises organelles, cells, microenvironments, tissues, organs, and systems. Mechanobiology factors commonly play a role at and below the tissue level, emphasizing local interactions among cells and between cells and ECM. It encompasses the micro-forces offered by ECM, the macro-forces produced by tissues, the dynamic forces such as interstitial flow, and the spatial structure of tissues (for example, villi). On the system level, chemobiology factors affect the entire body through the circulatory system, centering on remote interactions among tissues via signaling molecules and metabolism. Simulating mechanical biological factors is typically accomplished through specific matrix materials and microfluidic chamber structures, while controlling chemical factors often employs microfluidic channels. However, certain biological processes that impact the microenvironment level, such as nerve conduction-based neuromodulation, vascularization, and immune response, must be achieved through organoid assembloids. The various patterns of organoids provide avenues for research on development and disease as well as clinical and pharmaceutical applications.

system-level interactions among target cells and various environmental cues. Presently, organoid technology has been extensively applied in simulating the ECM, maintaining the immune microenvironment, establishing vascular microenvironments, and studying mechanobiology factors (Figure 2, Microenvironment).

Extracellular matrix (ECM). The ECM is mainly a macromolecular component that not only provides mechanical support but also creates a chemical environment for cells and tissues, thereby exerting significant influence in organoid-related research. In this context, the ECM plays a critical role in determining the physical and chemical characteristics of the culture environment, the cellular niche,⁴⁶ the transport of extracellular vesicles, and the storage and slow-release of growth factors to create spatiotemporal gradients. For instance, the physicochemical properties of the ECM impart biomechanical signals that regulate cell fate⁴⁷⁻⁵⁰ and influence cellular migration, invasion, and ecological niche establishment.⁵¹⁻⁵³ Moreover, the 3D structure formed by the ECM facilitates vesicle transmission and provides an ideal environment for studying signaling pathways.^{54,55} Consequently, numerous studies have focused on investigating the utilization of ECM materials from different origins or with distinct properties, which has emerged as a necessity in fundamental research on organoid formation.^{56,57} Furthermore, the complex

relationship between nerve cells and the ECM serves as a crucial mechanism for achieving neuromodulation. Nerve cells exert their regulatory function by releasing factors such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), thereby influencing ECM components and the developmental state of peripheral cells.^{58,59} Additionally, nerve cells secrete specific matrix proteases to modulate neurodevelopmental and regenerative processes.⁶⁰ The secretome also plays a role in regulating the regeneration and remodeling of the ECM and surrounding cells.⁶¹ The ECM, comprising relevant structural proteins including laminin, supports the normal development of nerve cells while guiding their growth and the formation of neural networks by controlling regional growth factor components.^{62,63} Moreover, it can modify the electrophysiological characteristics of nerves through its effects on ion channels.⁶⁴ Conversely, aberration in ECM components significantly impacts the connectivity and function of nerve cells, as observed in the case of β -amyloid protein in Alzheimer's disease.^{65,66}

These observations underscore the pivotal role of the ECM as the foundation for cell-to-cell interactions and the essential foundation for neural development. Understanding the significance of the ECM points us towards designing, implementing, and regulating multi-organoid interaction models centered around neural systems.

Neural-centric System Concepts

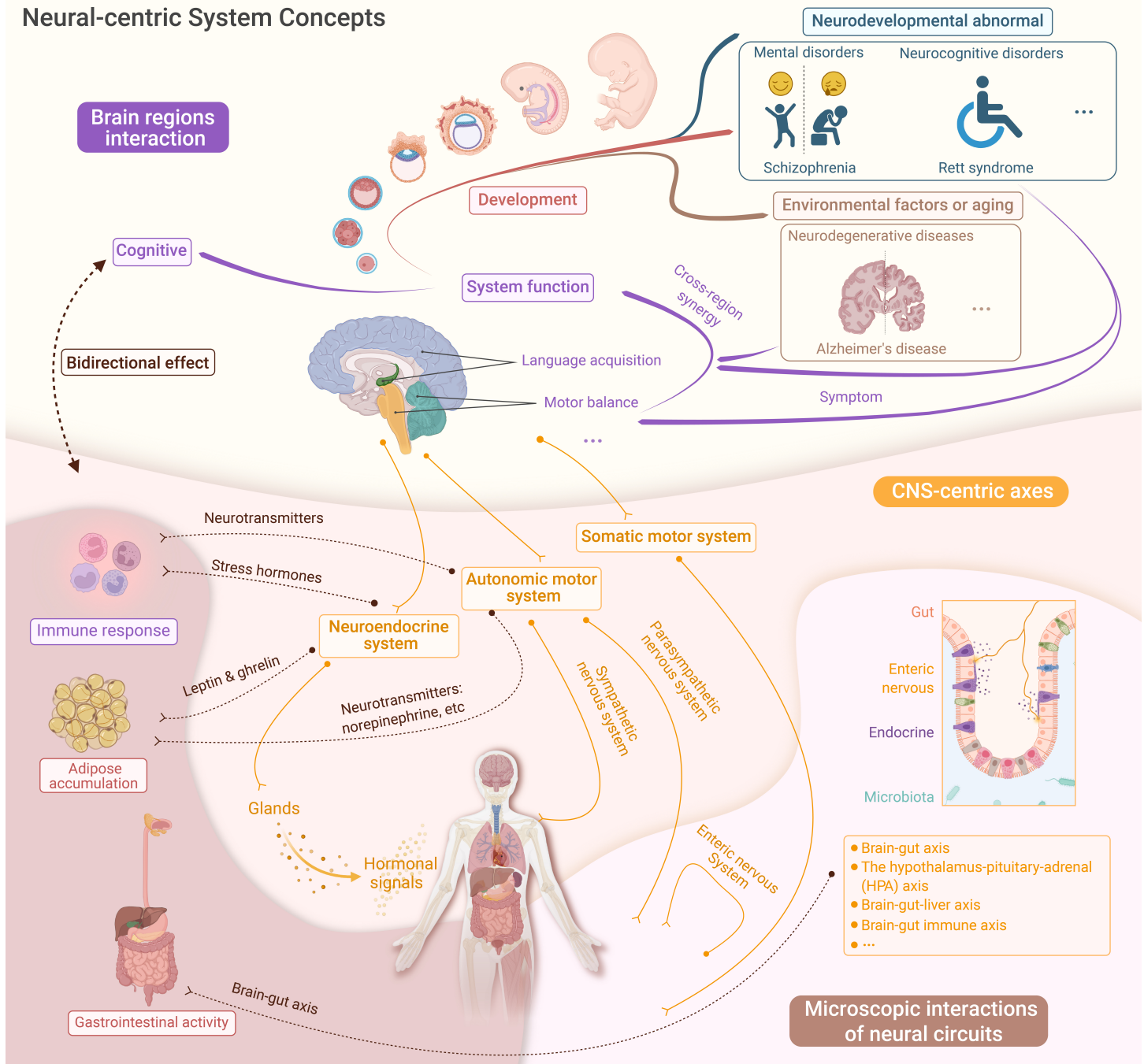


Figure 3. Neural-centric system concepts The nervous system-centric perspective is founded on the coordination between different brain regions and their regulation of various bodily functions through neural pathways. Efficient connectivity and cooperation between different brain regions are dependent on normal development, allowing cross-regional cooperation, while enabling advanced functions, such as language acquisition, motor balance, and cognition. Neuro-related diseases can result from abnormal development, environmental factors, or advancing age, and symptoms of such diseases are frequently cross-regional and can adversely affect the coordination of the system. The regulation and feedback between the brain and peripheral organs happen through neuroendocrine mechanisms or nerve conduction, with multiple ways of coexisting for some functions. For instance, leptin and ghrelin have neuroendocrine effects in regulating fat accumulation, while norepinephrine functions as a neurotransmitter with neuroconduction effects in this process. And the brain-gut axis encompasses the systematic role of the gut, enteric nervous system, endocrine, and microbiota.

Mechanobiological microenvironment. Compared to strategies applying small molecules or growth factors to guide the differentiation and organization of corresponding organs or tissues,⁶⁷⁻⁷⁰ the mechanical factors involved in organoid development and maturity are less investigated. It has been well reported that organoids are more permeable and mature by dynamic active infusion of nutrients or chemicals in culture⁷¹⁻⁷³ rather than passive diffusion. These, together with the aforementioned interstitial flow factors, and flow-induced shear stress constitute the application of flow-mechanobiological environment control. Analogously, our brain consumes 15% of total body glucose and receives 15% of the blood supply from the heart.⁷⁴ Blood supply

and circulation of cerebrospinal fluid into the brain or medium infusion into organoids create a fluidic impact on the living tissues. Under such situations, mechanosensitive ion channels are of assistance for cells to cope with shear force and hydraulic pressure to maintain their homeostasis.⁷⁵⁻⁷⁷

From the flow to the solid, the specifically designed microfluidic spatial structures like chambers were also used to study the mechanical effects in organoid development.⁹ Catering to the asymmetric polarization phenomenon that exists widely in human development, some studies have been devoted to controlling the shape and polarity of organoids via mechano-regulation. Examples include using ECM modulation to screen the relation-

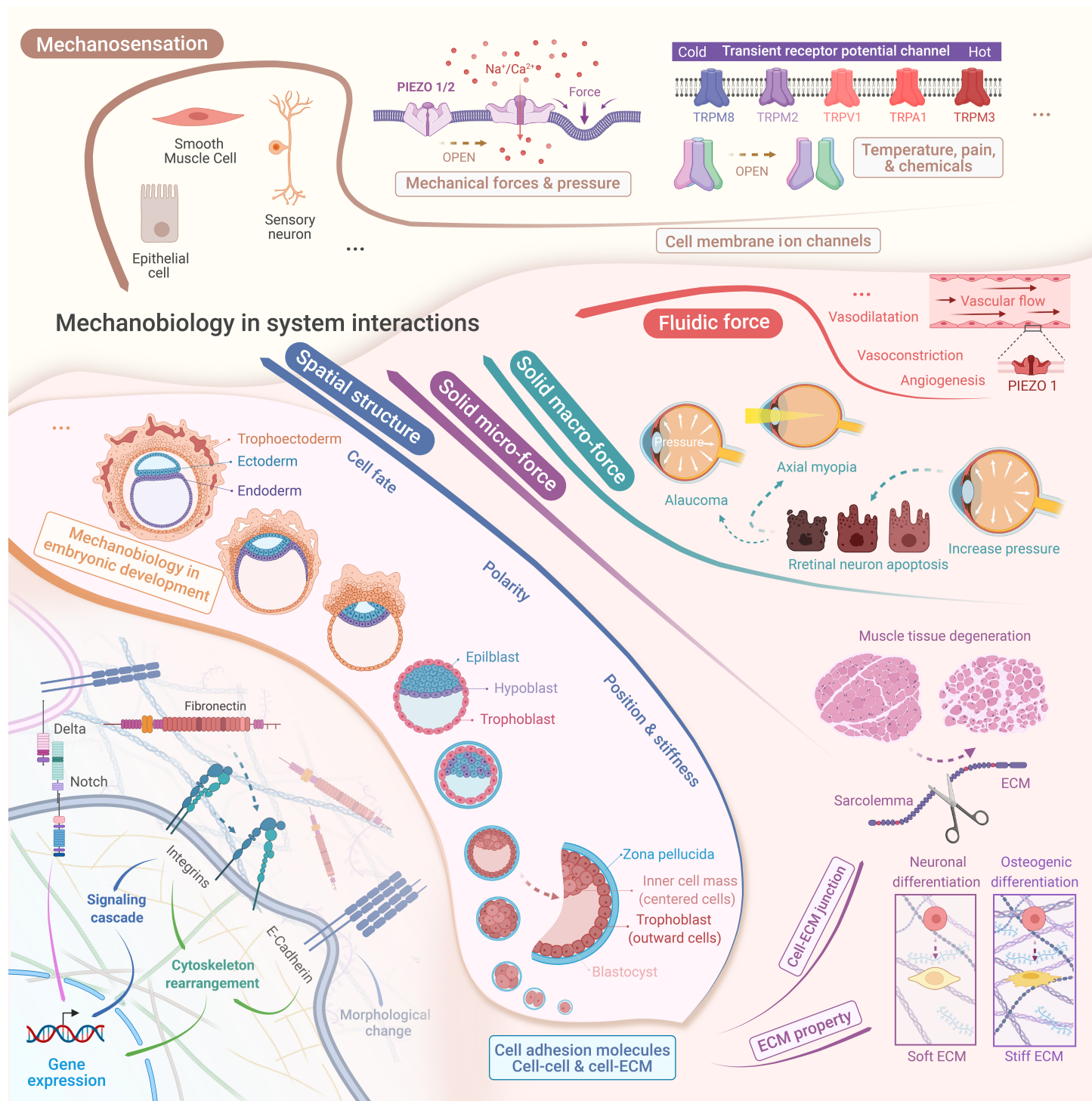


Figure 4. Mechanobiology in system interactions Cell adhesion molecules and mechanosensitive ion channels are the two primary ways through which human cells detect mechanical information. For instance, during embryonic development, cells sense their position (niche) and ECM properties through cell adhesion molecules, which generate polarity and determine cell fate. The human body perceives touch, cold, and heat through mechanosensitive ion channels present in the cell membranes of epithelial cells, sensory nerves, etc. Mechanobiology at the tissue and organ level can be categorized into static spatial structures, static solid micro-forces, dynamic solid macro-forces, and dynamic fluid forces. These categories play crucial roles in determining cell differentiation, tissue development, and function, as well as organ homeostasis and pathology.

ship between early neural tube morphology and the ECM condition,⁷⁸ and to tailor their polarity reversal in airway⁷⁹ and gastrointestinal organoids.^{80,81} It is possibly expected to achieve artificially controlled morphogenesis through predefined mesenchymal condensate patterns⁸² or matrix composition,⁸³ demonstrating the application of space-mechanobiological environment factors, in organoids used to study developmental processes. Studies that investigate the integration of multi-modal stimuli have shown that mechanical cues can be experimentally manipulated. This is achieved by combining mechanical cues with biochemical signals and transducing biomechanical

signals through biochemical transcription factors.⁸⁴ For example, the F-actin cytoskeleton can be inhibited by the molecular motor myosin II inhibitor Blebbistatin, the myosin light chain kinase selective inhibitor ML-7 hydrochloride, the Rho-associated coiled-coil kinase inhibitors Y-27632 and C3 Toxin, the Small Molecule Inhibitor of Formin Homology 2 Domains (SMIFH2), and the actin-cytoskeleton disruptors Latrunculin A and B,^{85,86} and the capping and severing proteins such as CapZ, cofilin, and gelsolin.⁸⁷ The F-actin cytoskeleton is a network of protein filaments important for cell structure and movement. By inhibiting components of this cytoskeleton, the activity of YAP/TAZ,

System View of Organoid Derivatives

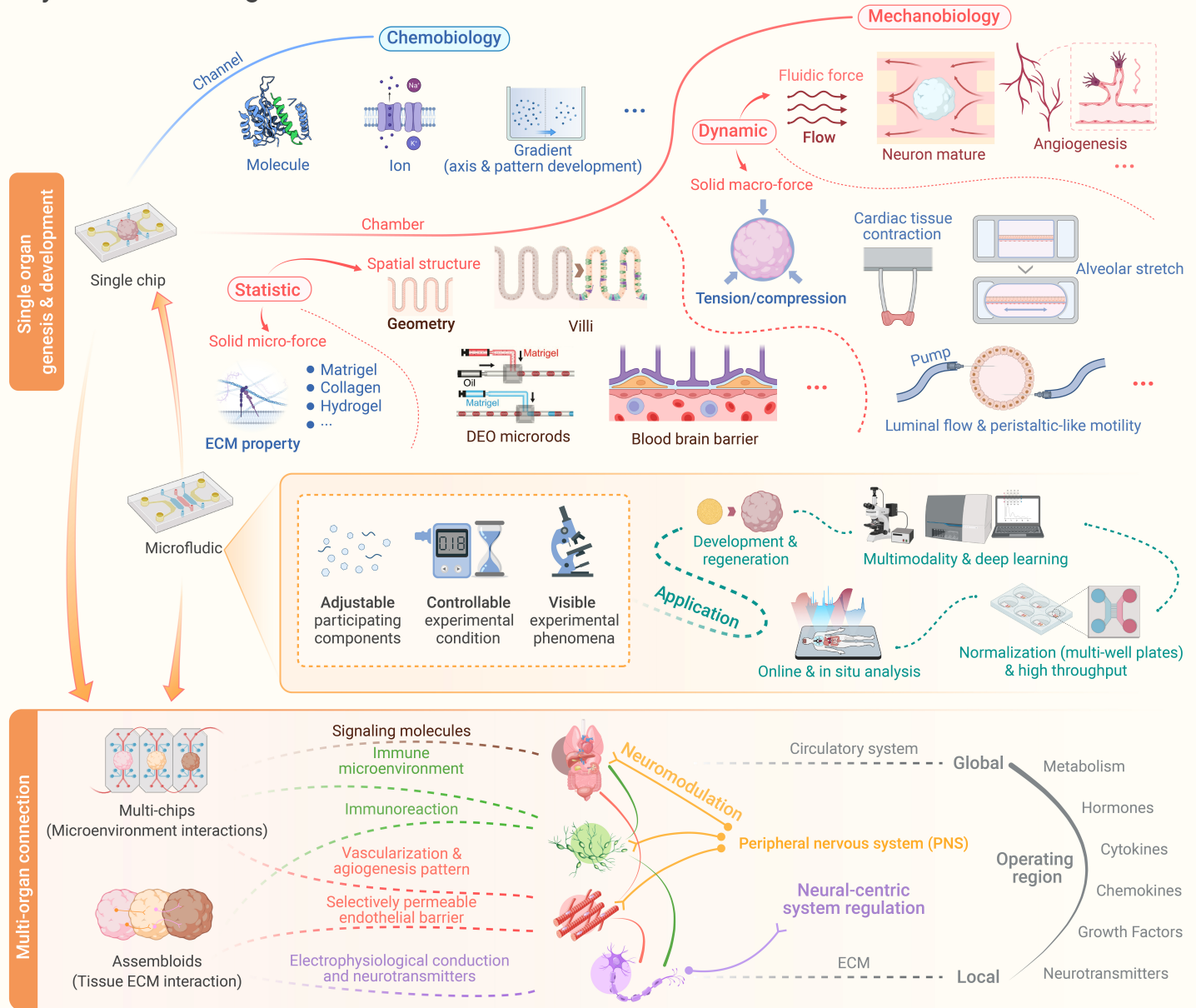


Figure 5. System view of organoid derivatives Depending on whether they consist of single or multiple elements, organoid derivatives can be divided into single-chip for single organoid development or multi-chip and assembloids for multi-organoid interaction. Single-chip facilitates the development of single organoids in two dimensions: chemobiology and mechanobiology. At the chemical level, precise regulation of molecular concentration and gradient control can be achieved. In terms of mechanobiology factors, it can simulate the ECM, structure, solid force, and fluid force for organoid development. Multi-chips enable the connection of multiple organs and can realize a series of global interactions such as signaling molecules and the immune microenvironment. Assembloids can achieve local interactions such as nerve conduction and immunoreaction in the microenvironment. This corresponds to the neural-centric system regulation, circulatory system, immune system, and peripheral organ interaction in the human body. The popularization of microfluidic in organoid derivatives has brought the advantages of adjustability, controllability, and visibility, and has enabled applications in the fields of development, multimodal, high-throughput, in-situ analysis, etc.

which are proteins involved in cell growth and differentiation, can be modulated.⁸⁴ These are some of the examples of how the synergistic effects of combining mechanical stimuli with biochemical cues can result in a better understanding and distinction of signals coming from the mechanobiological microenvironment.

Furthermore, a variety of hydrogels, each with specific physical properties, have been utilized to exert compressive forces upon cells or organoids.^{88–90} Recent studies have showcased a range of stress-relaxed synthetic hydrogels, which have been found to enhance organoid morphogenesis through reversible hydrogen bond-mediated dynamic rearrangement.⁹¹ Advancements in biomaterial engineering have led to the development of biomimetic scaffolds that closely mimic or diverge from the native tissue architecture. For example, engineered hydrogels can be adjusted for degradability, and

include controlled mechanical cues such as signaling molecules, and/or controlled mechanical properties, such as hydrogel stiffness, confinement, and elastic and viscoelastic properties, provided by hybrid and synthetic materials.⁹² Hydrogel mechanical properties were tuned to simulate healthy and stiffened cardiac tissue, where the role of the transient receptor potential vanilloid 4 calcium channel and regulation of myofibroblast activation and proliferation in valvular interstitial cells through mechanosensitive pathways was demonstrated.^{93,94} The effects of the mechanobiological microenvironment are particularly evident in 3D space. For example, 3D co-cultured cardiomyocytes and fibroblasts recapitulate synchronous beating and are effectively used to capture the transformation from healthy tissue to cardiac fibrosis, while 2D culturing essentially downgrades the cells' ability to beat.⁹⁵ Hence, applying these techniques and engineered materials enables the

creation of 3D culturing supporting structures that include organoids, which recapitulate the complex microenvironment of *in vivo* tissues.⁹⁶ However, in environments replete with diverse mechanical forces that influence cells in nuanced ways, the role of mechano-transduction cannot be overlooked in tissue development. For example, the contraction of classical cardiac tissue augments its maturation,⁹⁷ and glands proliferate within the stromal embeddings,⁹⁸ emphasizing the impact of mechanical forces on biological development. Advancements in dynamic modeling have enabled the simulation of complex mechano-biological interactions that occur between materials and biological entities. Hydrogels, for example, can be used to encapsulate cells and organoids.⁹⁹ The incorporation of 3D traction force microscopy and computational models that simulate variables such as traction stress can enhance our understanding of mechano-sensing pathways by visualizing modifications in the microenvironment.⁹²

Mechanosensing is not limited to the local tissue microenvironment. Studies on immunotherapies have shed light on how the role of mechanobiology expands at a systemic level.⁹² For instance, the chimeric antigen receptor T cell, widely known as CAR T cell, is actively investigated and used for non-solid cancer treatment, such as blood cancer malignancies.¹⁰⁰⁻¹⁰³ Studies have shown that unlike freely delivered T cells, CAR T cells that are confined in hyaluronic acid hydrogels retain more therapeutic potency, demonstrating less T cell exhaustion that is caused by persistent exposure to an antigen that resides in a tumor.^{101,104,105} The robustness of these cells may be attributed to their physical barrier rather than biochemical inhibitory signals that would prevent exhaustion. This highlights the role of mechanistic effects in biology. For example, Agrin's presence in the wound microenvironment is a prime example of how mechanical cues can profoundly influence cellular behavior. Agrin enhances the detection of mechanical cues by augmenting a series of biomechanical properties, such as stiffness and traction stress, in keratinocytes, which in turn promotes efficient wound repair.¹⁰⁶

The deployment of moderate compressive force, facilitated by alginate hydrogel encapsulation, onto cerebral organoids further highlights the importance of mechano-transduction in biological maturation, particularly in advancing neural maturation while promoting cellular proliferation.^{90,107} The above studies demonstrate the impact of using materials with different physical and spatial characteristics to regulate mechanical forces/mechanobiology environment from the microscopic to the macroscopic. In addition, the combination of microfluidics with droplet engineering produces organoids with diverse mechano-microenvironments. This results in the production of droplet-engineered organoids (DEOs) with specific sizes, shapes, structures, and patterns.¹⁰⁸⁻¹¹⁰

In general, although the difficulty of simulating the complexity of the mechanical environment *in vivo* has hindered the study of the actual development or regeneration process of organoids cultured *in vitro*, the flexible application of organoids with microfluidics and synthetic material technology is expected to achieve system integration and single organ independently development coexist. Then, through the control of flow, space, and microscopic/macroscale force mechanobiological factors, it is expected to personalize specific interaction models on the basis of better single-organoid development.

HUMAN BODY SYSTEMS AS A REALISTIC REFERENCE

Based on the current review of organoid advancements, it is evident that the primary focus of organoid research is on modeling the histology, pathology, function, and therapy of specific tissue regions. Chemobiological regulating factors have been discovered and developed for this class of studies, and are continuously advancing via cohesive efforts from biologists and chemists. However, despite some advances, the incorporation of system concepts in organoid modeling, such as neuromodulation and mechano-modulation, has been largely not implemented. This is mainly due to a lack of expertise and the challenges of inducing higher complexity. The regulation of mechanobiological factors is an essential aspect of the neural-centric biological processes' functioning, inseparable from the regulation of the microenvironment. For instance, the stiffness of ECM plays a role in regulating neural development.^{111,112} External mechanical factors, such as the regulation of macroscopic mechanical forces guide neural development⁹⁹ and patterning,^{113,114} and the design of the chamber structure in the microfluidic-

chip facilitate neuronal and vascular interactions (including the blood-brain barrier model)¹¹⁵ and the interactions within multi-source assembloids (such as neuro-immune interactions). Therefore, in future system organoid modeling development, it is crucial to discuss neural centralization and mechanobiological factors together.

Neural-centric system concept

The human nervous system, with the brain as its focal point, orchestrates a vast array of the physiological processes within the body. The collaborative dialogues that occur within the brain's complex networks not only underlie advanced cognitive functions but also extend bidirectionally to engage with other organ systems, thereby participating in myriad developmental and pathological processes. Therefore, in simulating important physiological and disease processes, it is necessary to introduce the simulation of the interaction between the relevant brain and peripheral organs in the dynamic process. Then embracing a systems-based perspective, particularly accentuating neuromodulation, empowers a nuanced exploration of the sophisticated interplay among diverse brain regions while recognizing the paramount role of the brain in forging and maintaining connections with peripheral organs. This nuanced intercommunication is mediated through pivotal axes such as the brain-gut axis, the hypothalamic-pituitary-adrenal (HPA) axis, the brain-gut-liver axis, and the brain-gut-immune axis. A meticulous investigation of these axes unveils invaluable insights into the complex mechanisms that facilitate communication and co-ordination between the brain and other critical organs. Such understanding paves the way for enhanced disease therapy, drug discovery and development, and healthcare management, approached from more comprehensive and precision-oriented perspectives. (Figure 3)

Brain region interaction and brain-organ interaction. As an important part of the central nervous system (CNS),¹¹⁶ the brain contains different interconnected brain regions¹¹⁷ (such as the cerebral cortex, hippocampus, and brainstem) and regulates organs through the peripheral nervous system (PNS).¹¹⁸ These connections are the basis for sensation, movement, and cognition during human body development and function.¹¹⁹

The realization of brain function relies on the synergy of different brain regions.¹¹⁶ Although separate studies of individual brain regions can effectively reveal their functions, the interaction mechanism across regions is often ignored, thus missing a comprehensive understanding of the brain's electrophysiological and cognitive functions. For example, the cerebellum and brainstem are involved in maintaining motor balance,¹²⁰ while the cortex and hippocampus contribute to language acquisition.¹²¹ However, the intrinsic self-organization exhibited during their development, along with their inherent collaborative mode of operation in executing functions, necessitates further detailed investigation. Moreover, the analysis of the entire brain system's functionality might require adherence to spatial patterning.¹²² This necessitates a high degree of customization and maneuverability in the experimental model employed, for which organoids present notable advantages.

The maturation and connectivity of brain regions depend on the typical developmental trajectory: from the initial division of specific regions in the embryonic stage, to further vesicle formation and inter-neuron migration across regions within the brain,^{38,123} until final maturation in the postnatal period.¹²⁴ Failure to follow this process often results in aberrant neurodevelopment and dysfunctional interactions leading to neurodevelopmental diseases containing mental disorders (such as schizophrenia)¹²⁵ or neurocognitive disorders (such as Rett syndrome).¹²⁶ In fact, both neurodevelopmental diseases that originally came from abnormal development processes like genetic mutation, and neurodegenerative diseases caused by environmental factors or aging can affect multiple brain regions. For instance, Alzheimer's disease primarily affects the cerebral cortex and hippocampus,¹²⁷ which include symptoms that begin with memory loss and can lead to difficulties with language, decision-making, etc.¹²⁸ Therefore, establishing a multi-regional systematic model is a rational necessity for deconstructing brain diseases. (Figure 3, Brain regions interactions)

The CNS receives neural and endocrine information from peripheral organs and dispatches commanding signals to facilitate homeostatic balance. The signals received from the surrounding environment, such as vision, auditory sense, tactile sense, and proprioception, are transmitted by individual nerves

e.g., the optic nerve, vestibulocochlear nerve, dorsal column-medial lemniscal pathway, and the spinothalamic tract. The CNS transmits signals via the autonomic nervous system (including sympathetic and parasympathetic nerves) and the neuroendocrine system to the visceral organs and glands to coordinate organ functions. This could be the basis for understanding why stress, depression, and anxiety significantly affect the human body and health. For instance, metabolism will change under stress, and gastrointestinal (GI) peristalsis will be abnormal,¹²⁹ also, bone-derived protein, lipocalin-2 (LCN2), can penetrate the brain-blood barrier and bind the melanocortin 4 receptor on hypothalamic neurons, thus activating the anorexigenic (appetite-suppressing) pathway.¹³⁰ In turn, the immune responses, adipose accumulation, gastrointestinal activity, etc., pose effects on the brain, inducing changes in mood and cognitive ability (Figure 3, Brain-peripheral organ association).¹³¹ Leptin is a hormone produced by white adipose tissue,¹³² which is released in the circulatory system and reaches the arcuate (ARC) and paraventricular (PVN) nuclei located in the hypothalamus. The BDNF positive neurons are activated and innervate white fat tissue to promote its conversion to brown adipose tissue.^{133,134} The direct connection between the brain and gastrointestinal tract mainly relies on vagal nerves, whereas the indirect association is executed by the endocrine systems, referred to as the CNS-centric axes. (See details in CNS-centric axes).

CNS-centric axes. When examining the cause-and-effect relationship of brain activity in development and disease, it is crucial to adopt a systematic approach and investigate its intricate connection with the body's "second brain" – the enteric nervous system,¹³⁵ which includes the gut and associated microbiota. This bidirectional communication, known as the brain-gut axis,¹³⁶ encompasses brain-gut-microbiota-immuno-endocrine interactions and the gut-liver-brain axis system (Figure 3, CNS-centric axes).¹³⁷

The brain-gut axis is a broad term that concentrates on the multichannel communication between the brain and the gastrointestinal system. This communication typically involves several pathways. One such pathway is the neuronal route, which connects the CNS and the enteric nervous system via the vagus nerve, utilizing neurotransmitters.¹³⁸ Another pathway is the endocrine system, which may involve the microbiota¹³⁹ and their metabolites, such as short-chain fatty acids.¹⁴⁰ Immune pathways are also crucial, involving but not limited to dendritic cells, macrophages, mast cells, and other immune cells, which continuously interact with the enteric nervous system through toll-like receptors and other mechanisms. These interactions play crucial roles in maintaining homeostasis and responding to infections, allergies, or inflammatory conditions. Molecular signals including cytokines and neuropeptides facilitate this bidirectional communication, underlining the importance of the neuro-immune axis in gut health and potential therapeutic interventions for inflammatory diseases.¹⁴¹

This intricate interplay between neural and immune responses is especially significant considering the gut's role as a key immune organ.¹⁴² Interactions among intestinal neurons, immune cells, and microbes, as well as inflammatory crosstalk between the gut and the brain, have been the subject of extensive research.^{141,143} Both innate and adaptive immune cells participate in these pathways, further underlining the complexity of these processes. Interestingly, there are shared functional and molecular characteristics between the blood-brain and gut vascular barriers. In both locations, local immune responses are mediated by glial cells. In the enteric nervous system, both neurons and glial cells actively contribute to the immune functions of the intestine. For instance, enteric glia aid in protecting the intestinal barrier by releasing GFRA1 ligands. These ligands, interacting with the RET receptor, subsequently trigger the production of interleukin-22 (IL-22) through the activation of ILC3 cells.¹⁴⁴ In a similar vein, the CNS employs astrocytes to safeguard the blood-brain barrier.^{145,146} As inflammation continues, damage to both the blood-brain and gut vascular barriers can occur, leading to a cascade of adverse events. For example, lipopolysaccharides from bacterial walls can leak from the gut to the brain, sparking neuroinflammation and directly leading to cognitive impairment, as evidenced in murine models.¹⁴⁷ As for the neurons, in the context of Parkinson's disease, intestinal alpha-synuclein aggregation can translocate to the CNS via the vagus nerve, activating T cells and microglia, leading to further immune recruitment and alterations in the dopamine circuit. It is important to note that many microorganisms, their metabolites, and their working mechanisms are yet to be char-

acterized, and their influence on immune upregulation or downregulation needs further exploration.

Recent research further extended the brain-gut-axis to the brain-gut-liver axis. For instance, fasting in mice has been shown to activate hepatic autophagy, a process of liver cell renewal.¹⁴⁸ Concurrently, the hypothalamus activates agouti-related protein (AgRP) neurons, linked to a slower metabolism and increased food intake. This activation induces changes in the phosphorylation of autophagy regulators, promotes ketogenesis, and elevates corticosterone levels in the blood. Remarkably, reduction in hepatic glucocorticoid receptor expression dampens the activation of hepatic autophagy that depends on AgRP neurons. This study, therefore, establishes a direct link between brain neuroplasticity and hepatic autophagy, as well as metabolic adaptation.¹⁴⁸ The importance of the brain-gut-liver axis is further emphasized in a study reporting that hepatic vagal sensory efferent neurons indirectly sense the gut microenvironment.¹⁴⁹ Surgical and chemical interventions at the hepatic afferent level resulted in decreased aldehyde dehydrogenase expression and retinoic acid synthesis by intestinal antigen-presenting cells, leading to reduced colonic pT_{reg} cells. This pivotal study reveals the systemic interdependence between neurons, the gut, the liver, and immunity.¹⁴⁹ Understanding the aforementioned systematic interaction is crucial in decoding numerous diseases, particularly those impacting mood and behavior, and those linked to neurodegenerative conditions. Multiple studies have highlighted a connection between the interdependence of intestinal microbiota and neurophysiological function in both human and non-human models. For instance, variations in gut microbiota composition have been associated with neurological disorders. Although most insights come from animal models, the evidence suggests a complex interplay involving multiple biological systems, with the gut microbiota affecting neurophysiological functions, thereby indicating a potential area for therapeutic interventions in neurological disorders (Figure 3, Bidirectional effect).¹³⁶

The Hypothalamus-Pituitary-Adrenal (HPA) axis is a hormonal cascade directly responsible for the body's stress response. This process starts with the hypothalamus stimulating the pituitary gland to produce adrenocorticotropic hormone (ACTH) through the release of corticotropin-releasing hormone. ACTH, representing endocrine signaling, then circulates to the adrenal glands triggering cortisol production.¹⁵⁰

In response to stress, the hypothalamo-pituitary-adrenal (HPA) axis is activated, leading to the secretion of cortisol by the adrenal glands. The released cortisol then acts to elevate blood sugar levels and modulate the immune response.¹⁵¹ Furthermore, the adrenal glands produce hormones like adrenaline and noradrenaline, slowing intestinal activity.¹⁵² Chronic elevation of cortisol/corticosterone levels leads to increased anxiety and disrupts allostasis.¹⁵³ This disturbance alters the gut microbiota¹⁵⁴ via stress-related conditions like anxiety, depression, and irritable bowel syndrome. In a study, researchers found that introducing bacteria from specific pathogen-free mice to germ-free mice at early stages partially normalized the HPA stress response in germ-free mice. This same effect was achieved by exposing mice to *B. infantis*, thus reducing their exaggerated stress response. Conversely, move-space restrained germ-free mice exhibit lower expression of brain-derived neurotrophic factors (growth factors) in the cortex and hippocampus.¹⁵⁵

Notably, the cross-talk between the HPA axis and the gut is dynamic and interactive. This interaction is established during the early stages of mammalian development and significantly influences the composition of the microbiota and the development of the nervous system. For instance, early prenatal stress can interfere with the natural microbial transmission from mother to male offspring, causing long-lasting effects on the baby's metabolism, physical health, cognition, and behavior. The vaginal microbiota transfer from the mother to babies delivered by cesarean section in natural birth can partially mediate the effects of prenatal stress on gene expression in the hypothalamus, as observed after chronic stress in adulthood.¹⁵⁶ One study highlighted the role of gut microbiota in mediating gut-brain signaling, showing that chronic ingestion of a specific *Lactobacillus* strain led to regional changes in brain GABA expression and reduced stress-induced HPA axis activation. This provides an example of how gut microbiota, influenced by diet, can modulate the HPA axis and potentially impact mental health.^{139,157}

Mechanobiology in system interactions

Mechanobiology has emerged as a critical field of study, elucidating the interplay between mechanical forces and biological processes in the context of development and disease. From single-celled organisms to complex mammals, cells constantly receive signals from their surroundings. These signals can be chemical or mechanical and are generated by the microenvironment. Advances in biotechnology have allowed for a greater understanding of the role of biological factors in embryonic development, organ function, damage repair, and disease progression.¹⁵⁸⁻¹⁶⁰ However, our understanding of the mechanical aspect of these processes is limited by current research techniques. Deciphering the roles of mechano-regulation factors in physiological and pathological conditions provides the foundation for modeling such roles in vitro by using advanced organoid system modeling techniques toward precision medicine.¹⁶¹

Mechano-transduction in development. Mechanical forces can trigger changes in intracellular chemical patterns and signaling cascades. For example, during mammalian embryonic development, cell adhesion molecules inform cells of their contacts with other cells. This leads to changes in cell shape, polarity, and cytoskeleton rearrangement, which in turn tunes the stiffness (or more precisely, viscoelasticity) of cells and promotes alternative gene expression patterns. The fate of cells is determined by their mechano-properties, such as polarity, stiffness, and position within the embryo. The cells outward will develop into trophoblasts, whilst the centered cells will become the inner cell mass (Figure 4, Mechanobiology in embryonic development & Spatial structure).¹⁶²⁻¹⁶⁴ Mechanotransduction can be regarded as the conversion of mechanical stimuli into biological signals. This ability is highly conserved across species, indicating its critical role in biological evolution.¹¹¹ Mechanosensitive channels on bacterial membranes, such as MscL and MscS, help to balance osmotic pressure when the membrane is stretched.¹⁶⁵ In mammals, the transient receptor potential (TRP) channels, degenerin/epithelial sodium channel (DEG/ENaC), and PIEZO1/2 detect ion concentration change and deformation of the cell membrane. Upon activation, these channels allow ion flow, creating an electrical potential or triggering transcriptional changes, which are then relayed as tactile and auditory sensations. Other membrane-bound proteins, like receptors of integrin, collagen, laminin, etc., transduce mechanical information, including cell distortion, shear or compressive stress, and ECM stiffness to the intracellular protein components, such as Talin or F-actin, which then transmit the signal via FAK, Hippo or other signaling pathways to guide gene expression (Figure 4, Mechanotransduction & Cell adhesion molecules).^{86,88,111,166-171}

Within these signaling pathways, one of the key determiners of cell morphology, F-actin, directly interacts with angiomin (AMOT) family proteins and therefore, inhibits the subcellular localization of YAP/TAZ towards the nucleus to function as a transcriptional factor promoting cell proliferation.^{172,173} Going a step further, force, like fluid shear stress or compressive force, can be transmitted along F-actin filament directly to the proteins like Nesprin-1/2 and then Sun1/2 located on the nuclear membrane, and Lamin proteins binding at the inner side of the nuclear membrane, which will cause deformation of chromatin, exposure of previously transcriptionally repressed regions, and release of transcriptional factors, such as NF- κ B in immune response modulation, which subsequently modulates gene transcription and subsequent protein production to respond to cell deformation.^{112,174,175} Research indicates that mechanical cues can alter microRNA expression, impacting cellular functions like proliferation, differentiation, migration, and mechanical homeostasis - the tissue's ability to maintain consistent stiffness, tension, and recovery from mechanical stress.¹⁷⁶⁻¹⁷⁹ A study identified 122 families of microRNA that target 73 mRNAs associated with the mechanobiology of endothelial cells. These mRNAs encode proteins related to the cytoskeleton, contraction, adhesion, and the cell-assembled extracellular matrix (CAM). The findings indicate that a network of microRNAs may regulate CAM expression to ensure mechanical stability in tissues, thereby preserving the equilibrium of CAM expression when mechanical stress fluctuates.¹⁷⁷ Aging is an indispensable part of development. With aging, the mechanical properties of tissues change, which can affect mechanotransduction processes. This has implications for tissue function, the development of age-related diseases, and longevity studies. For example, in *C. elegans*, the extracellular matrix changes with age. The cuticular colla-

gen rapidly declines, adhesome levels remain constant, and proteases and protease inhibitors such as MMP/zmp, astacin metalloprotease/nas, cathepsin/cpr, and protease inhibitor/cystatin/cpi increase. This results in a stiffer and mechanically weaker extracellular matrix.¹⁸⁰

Mechanobiology in health and disease. As noted earlier, the ECM directly interacts with cells and regulates numerous mechanical and biological processes, which play a significant role in the development and progression of disease. These processes are essential for maintaining tissue homeostasis and overall organism health.¹⁸¹ For instance, the mechanical properties of ECM have been shown to influence stem cell differentiation, with softer matrices promoting neuronal differentiation and stiffer matrices promoting osteogenic differentiation,¹⁸² consequently, what chronic inflammation and tissue remodeling trigger diseases like chronic obstructive pulmonary disease and ECM components changes like amyloid beta (A β) protein deposition associated with neurodegenerative disease, such as Alzheimer's disease.¹⁸³ Additionally, the mechanosensitive Piezo1 ion channel has been implicated in the regulation of blood flow and vascular development. Alteration in intracellular calcium homeostasis mediated by Piezo1 channels has also been implicated in the pathogenesis of many cardiopulmonary vascular diseases (Figure 4, Fluidic force).⁷⁵ In skeletal muscle, a mutation in the dystrophin-associated protein complex in the plasma membrane impedes the transduction of mechanical force from ECM to muscle cells, resulting in abnormal activation of ERK1 and ERK2 signaling pathways in response to stretch, and therewith degeneration of muscle tissue (Figure 4, Solid micro-force).^{184,185} Furthermore, an increase in hydrostatic pressure in intraocular conditions creates a pathological mechanical environment, resulting in apoptosis of retinal neurons and finally contributing to glaucoma and axial myopia.^{186,187} These highlight the importance of understanding the mechanobiological aspects of cellular behavior in maintaining tissue health and function (Figure 4, Solid macro-force).

Mechanobiological dysregulation has been implicated in the pathogenesis of various diseases, including cancer, fibrosis, and cardiovascular disorders. In cancer, the mechanical properties of the tumor microenvironment, such as increased matrix stiffness and altered cell-cell adhesion, can promote tumor progression and metastasis.¹⁸⁸ For example, increased ECM stiffness has been shown to enhance integrin signaling and activate downstream pathways, such as the Rho/ROCK and PI3K/Akt pathways, leading to increased cell proliferation, survival, and invasion.¹⁸⁹ In fibrotic diseases, excessive deposition of ECM components and increased tissue stiffness can lead to organ dysfunction and failure and eventually result in higher risks of cancer occurrence. Mechanosensitive signaling pathways, such as the transforming growth factor-beta (TGF- β) pathway, play a crucial role in the development of fibrosis by promoting the differentiation of fibroblasts into myofibroblasts and the production of ECM proteins,¹⁹⁰ and can enhance the infiltration of chimeric antigen receptor (CAR) T cells aforementioned.¹⁹¹ Similarly, in cardiovascular disorders, such as atherosclerosis, altered mechanical forces, including shear stress and cyclic strain, can contribute to endothelial dysfunction, inflammation, and plaque formation.¹⁹² As mentioned above and in, mechanobiology section has implications in immune function, fibrosis, wound healing, stem cell therapies, and beyond.¹⁹³ For example, gene therapy targeting the collagen α 1 promoter using an adeno-associated virus serotype 2 vector with the miR19b transgene in hepatic fibrosis Sprague Dawley rats caused improvement in fibrosis conditions.¹⁹⁴ Therapies involving stem cells and specific mechanical stress induced on them⁸⁴ can promote stem cell differentiation toward cardiovascular cell types,¹⁹⁵ enhance myocardium regeneration, and specific migration.¹⁹⁶ In addition, mechanobiology co-develops with advances in material science, making it more accessible in healthcare. For instance, using a chitosan-PEG hydrogel to co-deliver CAR T cells with IL-15 in mice with retinoblastoma treated cancer while eliminating the usual vision loss⁹⁷ associated with T cell treatment.⁹² Understanding the mechanobiological aspects of disease progression may therefore provide novel therapeutic targets and strategies for the prevention and treatment of these disorders (Figure 4, ECM property).

MODELING FOR MULTI-ORGAN ASSOCIATION REALIZES MULTI-TERMINAL INTERACTION

Utilizing organoid technology to mimic system-level interactions as

observed *in vivo* presents challenges, stemming from an incomplete understanding of human somatic functions, along with limited manipulation techniques. However, organoid technology has a unique feature compared to animal models in that it can accurately regulate participating units, such as the regulatory axis and brain-region interactions, as mentioned later. In this manner, variables that are not related to the hypothesis can be separated to the greatest extent possible. This allows for the unification of the isolation and deciphering of dynamic biological processes. By gaining insight into complex neural-centric and mechanoregulation principles, there exists potential to advance organoid modeling techniques. These enhanced methods could simulate system-level interactions of identified key factors, thereby providing refined tools for precision and regenerative medicine endeavors. A comprehensive review has been conducted on the modeling methods of engineered organoids.⁴⁸ For example, the most common method of simulating ECM with hydrogel was discussed, including the impact of different biomaterial conditions⁹⁹ and manufacturing methods on organoid modeling. When modeling neuro-centric organoids, it is important to consider the microenvironmental requirements, parentage, consistency, and high-throughput requirements to evaluate the modeling methods of individual organoids. For instance, stem-cell derived assembloids can be used to generate cortical organoids that more closely resemble the functional tissues of the human cerebral cortex. Droplet-engineered organoids can partially recapitulate the biological processes of organs or diseases and have the advantages of high-throughput and consistent production.¹ There also exist various organoid-on-a-chip models, encompassing serial fluid environment models, semi-contact models like membrane co-culture, and direct fusion models known as organoid assembloids. Each model showcases a distinct set of attributes aligning with specific research design needs. This methodological diversity heralds a significant avenue for probing interactions within organoids (Figure 2, Organoid-on-a-chip & Organoid assembloids).

Organoids-on-a-chip

Over the past decade, microfluidic-based biochip technology, encompassing cell-based systems, engineered droplets, and organoids, has undergone dynamic advancement. Distinct from the conventional organoid cultures mentioned earlier, microfluidic technology has demonstrated its capacity to significantly enhance the maturation and development of individual organoids, facilitate the connectivity and interplay between multiple organoids, simulate mechanobiological conditions, and offer high-throughput capacity for screening and detection. For instance, in modeling single organs, microfluidics has exhibited exemplary capabilities across various organs, including the brain,^{9,52} spinal cord,¹⁹⁸ lungs,^{199,200} intestine,²⁰¹ blood vessels,^{202,203} kidneys,²⁰⁴ and heart.⁹⁷ The flow environment in microfluidic chips has often exhibited facilitatory effects on tissue development, such as the enhanced expression of cortical layer makers,²⁰⁵ and the structuring of tissues, such as the formation of microvascular networks.^{206,207} The nuances and intricacies of these advancements have been documented in related reviews (Figure 5, Mechanobiology).²⁰⁸⁻²¹⁰

Recent advancements in multi-organoids-on-a-chip have predominantly concentrated on investigating the connectivity and interactions between multiple organoids. A prevalent indirect contact method entails a shared fluidic environment, which simulates inter-organ communication within the circulatory system through soluble signaling molecules, typically achieved via specialized actuated fluidic conduits.^{211,212} This method boasts considerable versatility in the regulation of organ type, fluidic components, and sequencing, and harbors the potential for autologous co-culture communication,²¹³ sustained culturing,²¹⁴ immune environment establishment,²¹⁵ and modular assembly (Figure 5, Multichips).²¹⁶ It also allows control of the number of neurons through the fine tuning of the hydrodynamic resistance and cell deposition rate to recapitulate the heterogeneity of brain regions in terms of cellular density and number, leading to brain region-specific modeling.²¹⁷ Following the above discussion on interstitial flow to regulate angiogenesis followed by organoid vascularization,^{204,218,219} organoids-on-a-chip have also demonstrated capabilities in partially replicating blood vessel functions^{218,219} and influencing the activation of specific genes involved in neural differentiation and neurogenesis during spinal cord-chip development.²²⁰ Importantly, the proposition of establishing selective permeability via endothelial barriers,

through vascular flow and capitalizing on the inherent function of the endothelial barrier,²²¹ suggests the potential for employing specialized functional cells to achieve chip functionalities, heralding a promising avenue in the design of chip connectivity through compatible contact and contactless solutions. In fact, this is similar to what is advocated to establish neural-centered organoids-on-a-chip, because the nervous system has a compatibility that can hardly be achieved by artificial materials, and has the functions of regulation and representation. Utilizing the functions of human cells and organs in organoids-on-a-chip meets the requirements of their high compatibility and integration (Figure 5, Microfluidic chip). Albeit the advancements in organoids-on-a-chip technology, there is a lack of vascular and structural interactions in models like brain, cardiac, and liver organoids. This limitation hinders the full potential of these models in replicating the complex interplay of different organ systems in the human body.

In addition to leveraging microfluidic technology for tailoring fluid environments, microfluidic chip technology has been instrumental in simulating mechanobiological conditions. Researchers have observed that in the context of dynamic mechanical factors, beyond the aforementioned enhancement of angiogenesis^{204,219} and culture maturation due to fluidic environments,^{52,205} dynamic mechanical factors such as passive stretching of the culture substrate^{199,215,222} and active tissue stretching exhibit positive effects.⁹⁷ This mimics the dynamic environment intrinsic to internal organs, as exemplified by the passive stretching of alveolar structures due to air pressure variations,¹⁹⁹ and the active contractions of cardiomyocytes.⁹⁷ In contrast, under static conditions, the emphasis shifts towards simulating spatial environments, which involves utilizing constrained spatial structures to emulate processes like cerebral cortex folding,⁹ employing specially preset structures to replicate the ecological niches of bone marrow¹⁹⁸ or intestinal villi,²⁰¹ and integrating flow channels with distinct structural designs to guide the formation of specific vascular patterns.^{206,207} While the implementation of static mechanical factors primarily revolves around matrix regulation, recent advancements in pressure control within embryonic development studies^{223,224} hint at the imminent integration of static stress factors into organoids-on-a-chip technology. Ultimately, the goal is to enhance the use of organoids-on-a-chip by merging bioreactors with microfluidics, advancing the existing technology and progressing toward integrated engineering. (Figure 5, Mechanobiology).

Breaking away from the practice of microfluidic technology on specific models, from an engineering perspective, organoids-on-a-chip technology also has the advantage of the ease to achieve high-throughput and rapid multimodal detection. Technically, there are applications of morphological information and computational vision algorithms for the detection of organoid status (such as intestinal organoid development,⁶⁷ neuroectoderm organoid development,²²⁵ immune cell interaction,²²⁶ or cancer drug response),²²⁷ and use the non-contact method of the fluid environment to perform continuous in-situ chemical detection.^{228,229} Moreover, there have been related designs that are compatible with standardized well plates and have achieved multi-model versatility.²³⁰ This is a direction worthy of attention for the subsequent application of organoids-on-a-chip technology in drug development and clinical practice (Figure 5, Application). However, it has a notable limitation: the inability to emulate the direct physical contact between tissues, thus precluding the modeling of neural regulation through synapses, endothelial barriers, and tissue microvascular networks, including the blood-brain barrier.¹¹⁵ Despite this, organoid-on-a-chip inherently recapitulates advanced bioengineering techniques from organoids to using microfluidic physics to understanding mechanosensing, proving them to be a challenging yet worthy pursuit. For example, organoids with PKHD1 mutation phenotyping autosomal recessive polycystic kidney disease do not show signature transcriptomics with static culturing models. Yet, under an organoid-on-a-chip model subject to flow, it reveals phenotypes of distal nephron dilatation and corresponding pathways. Here, mechanosensing molecules RAC1 and FOS1, targeted by drugs, successfully suppressed cyst formation. This serves as an excellent example of demonstrating implications of mechanobiological processes and considering them as important factors in drug testing and discovery. Additionally, organoids-on-a-chip, serving as platforms, that are successfully modeling *in vivo* systems.²³¹ However, such limitations might be overcome by adopting the strategy of organoid assembloids.

Organoid assembloids

Compared with the design of organoids-on-a-chip based on microfluidics as bioreactors, organoid assembloids are a direct fusion technology. It has the characteristics of direct physical linking or integration and is expected to be combined with organoids-on-a-chip in the future. From the perspective of neural-centric architecture, we can analyze the forms of organoid assembloids with the brain as the interactive core. Firstly, organoid assembloids are created by mixing with specific cells, allowing for the pre-mixing of different cell types in the initial stage (such as vascularization of brain organoids)²³² or cell invasion after the maturation of the main organoid (such as glioma models).²³³ This organoid assembloids method has been demonstrated in the study of pathological aspects of microglia cells in neurodegenerative diseases.²³⁴ Then, by taking advantage of the tissue fusion strategy, assembloids between different brain regions, such as the thalamus and cortical layers,²³⁵ have been used to study the interplay between different brain regions, and several studies have focused on neuro migration and interaction between the ventral forebrain and the dorsal forebrain.^{34,236,237} Similarly, for neural connections and regulation in peripheral organs (such as neuromuscular), there are two common approaches: using homologous simultaneous self-organization²³⁸ and linear assembloids of three types of organoids, that is, cortex, spinal cord, and muscle, to generate the nerve trajectory to transmit neuronal impulses to control the distally fused muscle organoids (Figure 2, Organoid assembloids).²³⁹ Both methods demonstrate interplay and connectivity between regions. Likewise, the retinal organoid protrudes its axons to the assembled brain organoid and prolongs the growth and survival of the retinal organoid²⁴⁰ and the merging of brain organoids and blood vessel organoids facilitates the vascularization of neural tissue.²⁴¹ Furthermore, there is also research focusing on the study of peripheral organoid assembloids such as investigating integral multi-organ structure continuous patterning and dynamic morphogenesis through hepatic, biliary, and pancreatic structures.²⁴² Similar to the above, human cardio-pulmonary co-development can be constructed by simultaneous multi-lineage differentiation.²⁴³ These studies have demonstrated that in the practice of system interaction, the assembloid of multiple organoids has a positive effect on the development and function of monomers and partially reproduces the system function of the human body. (Figure 5, Assembloids)

DISCUSSION AND PERSPECTIVE

The current development and application prospects of organoid technology demonstrate rapid progression; however, certain limitations still need to be addressed. One such limitation is the restricted recapitulative ability of parental organs. To overcome this, a potential solution lies in the selective recapitulation of specific features tailored for specific applications. Based on the above review, this is essential for the production and application of both brain organoids and peripheral and immune organoids. Another challenge is the technology required to achieve system-level construction. As system-level interactions require simultaneous implementation of different types of connections: direct connections between organoids through the microenvironment of the ECM, connections through macroscopic fluid systems (such as the vascular system and endocrine regulation), and preparatory conditional interactions (such as immune reaction). This poses challenges for the design of separation and integration in microfluidic bioreactors for specific organ regions and surrounding circulation and immune systems. Current technologies for organoid vascularization focus on two methods: self-assembled pre-vascularized organoids²⁴⁴ and engineered vascular beds²¹⁸ for vascular invasion. However, their implementation in neuro-centric organoid models requires further consideration of specific requirements. Such as assembloids that promote development and diffusion or blood-brain barrier organoid models that explore specific structures. It is important to note that using specific chip structures can replace part of the vascular transmission structure to compensate for the longer distance modeling of the circulatory system.²²¹ This allows for a neuro-centric design that prioritizes ECM-level neural patterning over modeling of the circulatory and immune systems. This demonstrates that in future systematic modeling of organoids on a chip, how to provide the mechanobiological design in the chip to achieve the establishment of neural projection and regulation will be the central challenge. In terms of specific practice, taking the neural-centric organoid system model-

ing as an example, it may be a feasible method to guide the neural projections of brain organoids on a specific microfluidic chip structure to form a semi-assembloid with the organoids in other chambers. Alternatively, this could be achieved using artificial two-dimensional neuronal cultures as an intermediate.

Based on the above overview, it is necessary to examine the direction of organoid technology from the perspective of neural-centric systems by mapping organs through organoids to obtain a more comprehensive view of the human body. For example, the brain-gut immune axis analyzed in this article plays a significant role in the gut-brain axis conversation. Most studies have concentrated on physiological markers and biological disruptions that primarily cascade from the gut to the immune system and then to the brain. Given the strong interconnection between the gut and the brain through multiple pathways, particularly in a neuroendocrine manner, it would be intriguing to explore whether changes in the brain and neural circuits could trigger this cascade, for instance, by altering gut motility, permeability, and other factors according to metabolic or environmental needs dictated by the brain. These findings suggest that the brain-gut connection plays a pivotal role in the systematic interaction between the CNS and peripheral organs. Such systematic considerations should guide the modeling, study, and research of these models, as overlooking these substantial systematic connections risks obscuring our understanding of disease and functional biology. Moreover, in terms of specific technical implementation, neural cultures based on organoids-on-a-chip technology²⁴⁵ show the possibility of combining photon stimulation and multi-electrode array (MEA) electrophysiological recording.²⁴⁶ Combined with the practice of developing the autonomic nervous system in organoids,²⁴⁷ it is inevitable to pioneer a neural-centric multi-organoid-on-a-chip model that synergizes organoids into a cohesive framework for isolating and deciphering the hiding systematic neural regulation pattern in key axes, alike the aforementioned brain-gut and HPA axes. For the exploration of organoid derivative modeling, it is also beneficial to emphasize the evolution of a multi-organoid interconnected structure. This focus aims at the development of an organoids-assembloids-on-a-chip model that amalgamates the benefits inherent in these organoid configurations. This strategy offers advantages in adjustability, controllability, and visibility, promoting its application in development, multimodal, standardization, and online analysis. The channel design of the single chip can precisely control chemobiological factors. The chamber serves as the central site for simulating mechanical biology induction, incorporating both static and dynamic, spatial structures and mechanical conditions. Furthermore, achieving multi-organ interaction requires complementary simulations of organoid assembloids and multi-chip connections, enabling the emulation of system interactions ranging from local to global levels. For interactions within the tissue ECM, the typical form is an organoid assembloid, with examples including neurotransmitter and nerve conduction, and immune reactivity. At the microenvironment scale, effective communication is achieved through the coordinated action of multi-chips, facilitating the transmission of signaling molecules and maintaining a continuous supply of the immune system micro-environment. So, identifying the benefits of both models and integrating them is the fundamental challenge in the future of modeling organ-like systems.

Regarding the specific technical realization, we advocate the use of organoids-on-a-chip. First, the precision and maneuverability of the engineered microfluidic chip technology create the conditions for the formation of highly-ordered multi-organ interaction link models. Second, it provides a technical basis for the simulation of mechanobiological factors. Third, the high throughput and reproducibility provided by multi-well plates such as 96/384-well formats also promote the large-scale application of organoids in medicine and clinical practice.^{2,227,248} Due to its ability to seamlessly integrate and study interactions between different organs and drug responses, as well as its adaptability to fine-tune specific components included in the model to ensure precise control of variables, it offers a promising solution for the demanding requirements of high-precision quantitative research in the field of precision medicine. To establish a neuro-centric system organoid model, it is necessary to design the chip in a way that separates different channels and chambers while satisfying the spatial position within the possible range of nerve projection, and then consider the introduction of the circulatory system

and the immune system. However, current research lacks a universal systematic model, with limitations centered around the necessity of achieving a stable and controllable fluid environment within the chip and a reliable semi-connection structure between chambers to achieve neural projection. The integration of dynamic processes is limited by various factors, including the microenvironment of different organoids and the regulation of long-distance nerve projections. Microfluidic chips offer a unique solution to these limitations due to their customization and micro-scale high accuracy, and are expected to achieve the separation of different channels and chambers while satisfying the possible range of neural projections.

At the same time, heterogeneous germ layer origin, the mechanobiological environment, and limitations in diffusion (such as vascularization) pose molding condition issues that need to be resolved. Additionally, despite limited prior knowledge of human biology and diseases, advancements can be achieved by cross-validating our understanding of human development and disease processes through the development of organoids, and vice versa. Another constraint is the scarcity of accessible physiological and pathological data. For instance, electrophysiological detection suffers from limited 3D optical detection depth and low spatial resolution of electrode-based sensing. But as *in vitro* models, organoids have less invasive limitations, which pave the way for direct detection of electrophysiological signals. Despite these aforementioned limitations, the flexibility and controllability offered by organoids-on-a-chips and organoid assembloids are sufficient to aid in the discovery of effective solutions for future research. Considering the neural-centric system view, it is worthwhile to investigate the potential of assembloids-on-a-chip and multiwell-plates as standardized chip-carriers. The availability of a controllable, adjustable, and visually perceivable assembloids-on-a-chip model and the ease of well plate accessibility to existing engineering systems are essential requirements for the large-scale implementation of organoids in scientific research and clinical practice. Indeed, the organoids assembloids-on-a-chip model, with its high-throughput and vast data generation capability, coupled with enhanced biological relevance, holds promise as a foundational tool for AI-big data based development, drug discovery, and precision medicine. The bottleneck of AI in medicine chiefly lies in the lack of high-quality data, a gap that the organoid models are well-poised to bridge, thereby propelling the frontier of personalized medicine and novel drug development to new echelons.

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FUNDING AND ACKNOWLEDGMENTS

The work was supported by the National Natural Science Foundation of China (32371470 and 82341019), the Department of Science and Technology of Guangdong Province (2023B0909020003), the Natural Science Foundation of Guangdong Province (2021B1515020092), the Shenzhen Science and Technology Innovation Commission (RCYX20200714114736146), and the Cross-disciplinary Research and Innovation Fund of Tsinghua SIGS (No. JC2022007). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

AUTHOR CONTRIBUTIONS

S.M. and P.E.L. conceived and advised on this work. S.M. and T.J. provided a substantial contribution to the content and generated all figures. S.M., P.E.L., T.J., D.K., Z.W. and Y.Z. contributed to paper revising. All authors reviewed the literature and contributed to writing and discussion.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DATA AND CODE AVAILABILITY

Not applicable.

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