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Review Article

Mechanical force in organoid and organ-on-a-chip systems: Design principles, biological effects, and translational applications

Yi-Jie Li[1,2,§], Yu-Yan Zhong[1,2,§], Wei Wang[2]✉, Chen-Shuo Yu[1], Yang Zhang[1]✉, and Qiang Zhao[2]✉

[1] *Department of Rehabilitation Medicine, School of Acupuncture-Moxibustion and Tuina and School of Health Preservation and Rehabilitation, Nanjing University of Chinese Medicine, Nanjing 210023, China*
[2] *College of Electronic and Optical Engineering & College of Flexible Electronics (Future Technology), Nanjing University of Posts and Telecommunications, Nanjing 210023, China*
[§] *Yi-Jie Li and Yu-Yan Zhong contributed equally to this work.*

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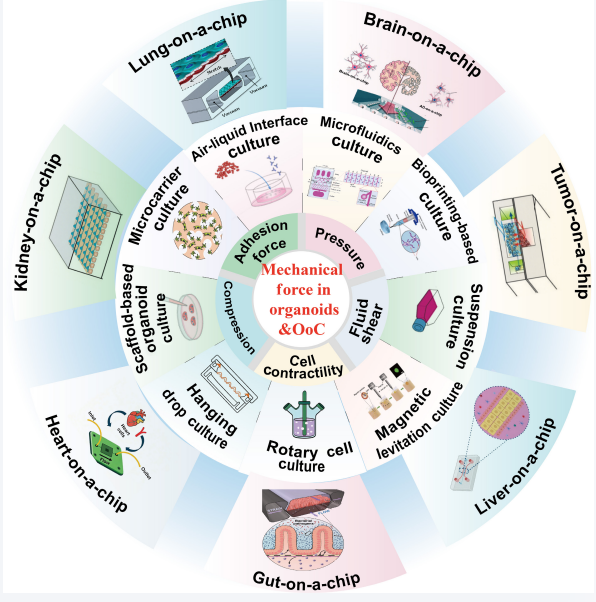
ABSTRACT: Organoid and organ-on-a-chip (OoC) are transformative *in vitro* platforms for biomedical research. A critical determinant of their physiological relevance is the faithful recapitulation of the native mechanical microenvironment, which governs cell behavior, morphogenesis, and maturation. Despite its recognized importance, a comprehensive review dedicated to the principles, design, and application of mechanical stimuli in these advanced systems remains limited. This review provides a systematic overview of this crucial aspect. We first elucidate the fundamental principles of mechanobiology, detailing how cells perceive and transduce mechanical cues such as fluid shear stress, substrate stiffness, and tensile strain. Subsequently, we analyze the implementation of mechanical control across diverse organoid culture methodologies, including scaffold-based, microcarrier, and advanced scaffold-free techniques. The review's core examines the application of engineered mechanical forces in various OoC models (e.g., lung, gut, heart, tumor), demonstrating how simulating physiological forces like cyclic stretching and peristalsis enhances biomimetic fidelity and functional maturation. Finally, we address key challenges and future prospects, including multi-scale stimuli integration, smart responsive materials, and mechanically coupled multi-organ systems. This work underscores the indispensable role of mechanical engineering in advancing organoid and OoC technologies for basic research and clinical applications. This review not only systematically synthesizes current progress but also proposes mechanobiological frameworks for the next generation of organoid and OoC systems.



KEYWORDS: mechanical forces, organ-on-a-chip, organoid culture, advanced fabrication, force on organs

1 Introduction

With the rapid advancements in biotechnology and materials science, replicating human organ structures and cellular compositions *in vitro* has become a prominent research goal. Organoid culture and organ-on-a-chip (OoC) systems have emerged as pivotal technologies for engineering such *in vitro* models. These platforms are increasingly demonstrating significant potential across diverse fields. Their applications include disease modeling, pharmacological screening, and personalized medicine.

Organoid culture refers to the three-dimensional (3D) cultivation of stem cells *in vitro*. These stem cells can be embryonic stem cells, induced pluripotent stem cells, or adult tissue-specific stem cells. Through processes of self-organization and differentiation, the cells

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✉Address correspondence to Qiang Zhao, iamqzhao@nuist.edu.cn; Wei Wang, wangwei29@njupt.edu.cn; Yang Zhang, yangzhang@njucm.edu.cn

form miniature organ-like structures, known as organoids. These organoids recapitulate some key structural and functional aspects of their *in vivo* counterparts. An OoC is a microfluidic device. It integrates miniaturized, fluidically perfused channels and chambers on a chip for cell culture. The goal is to recapitulate physiological functions and specific microenvironments. This includes simulating mechanical cues like fluid shear stress (FSS) from blood flow and physical deformation from respiratory movements. The evolution of organoid technology can be divided into three key stages. These are traditional two-dimensional (2D) culture, 3D organoid culture, and OoC integration. Early 2D methods used simple platforms like Petri dishes or flasks. However, they were limited in simulating the *in vivo* 3D microenvironment. This limitation often led to biased cellular behaviors and drug responses [1–3]. These approaches also lacked other critical features. They were missing diverse cell types, appropriate mechanical properties, chemokine crosstalk, and realistic fluidic conditions. Consequently, 2D methods could result in genetic drift during long-term passaging. This often led to unreliable research outcomes. To overcome these drawbacks, 3D organoid culture was developed. 3D methods enable the formation of tissue analogs with spatial structures. These structures more closely mimic *in vivo* architecture and functionality [4]. Organoids can be stably propagated long-term. However, they face several challenges. These include high variability, low maturity, and limited controllability, often due to static culture conditions. OoC technology aims to address these limitations. It does this by integrating organoid culture with advanced engineering techniques. Key techniques include microfluidics, dynamic environmental simulation, and multi-module integration. These platforms can simulate complex *in vivo* microenvironments. This simulation includes multiple cell types, tissue interfaces, biological fluids, and mechanical stimuli. The result is a micro-physiological system that better reflects human tissues and organs. In recent years, significant progress has been made in OoC development. This progress has led to the generation of various engineered organoids. Prominent examples include models of the lung, heart, kidney, liver, and intestine.

In the current research landscape, the influence of mechanical factors on organoid and OoC systems is garnering increasing attention. This is due to their pivotal role in regulating cellular behavior, tissue morphology, and functional expression. As a key external stimulus, mechanical forces are perceived by various cellular mechano-sensors. These sensors then transduce the physical signals into biochemical responses. These responses ultimately govern processes like protein expression and tissue morphogenesis [5]. Understanding this mechanobiological interplay is essential. It is key to capturing the fundamental characteristics of life *in vitro*. To this end, organoid culture and OoC technologies employ divergent fabrication strategies. Organoid culture focuses on self-organization, while OoCs emphasize engineering precision. However, they converge on a shared objective. This objective is to recapitulate physiologically relevant tissue microenvironments. Their synergy, therefore, necessitates a comprehensive integration of the biomechanical landscape. This landscape includes critical factors like fluid shear stress, cyclic tensile strain, and matrix stiffness. These factors are indispensable for ensuring model fidelity. Thus, the fusion of these two technologies provides a unified experimental platform. It allows researchers to decipher the intricate roles of mechanical stimuli in both physiological and pathological processes.

The history of applying mechanical forces in organoid culture reveals a clear transition. It has moved from simple static models to systems incorporating rudimentary mechanical assistance. Initial organoid culture systems primarily focused on chemical signaling and nutrient supply. However, they provided limited control over the physical mechanical environment. This changed following a key discovery. Researchers found that mechanical signals significantly impact cell differentiation and tissue morphogenesis. Consequently, they began integrating simple fluidic and solid mechanical factors. For example, devices like spinner flasks and orbital shakers have been employed. These tools simulate *in vivo*-like flow conditions. Their primary purpose is to enhance convective transport and nutrient exchange [6]. While these methods improved organoid scale and structural complexity, they offered limited control. Specifically, their spatiotemporal control over mechanical vectors was insufficient. Consequently, traditional organoid cultures often suffer from issues like high variability, low maturity, and limited controllability. These problems stem from the relatively uncontrolled or static growth conditions.

To address these inherent limitations, OoC technology emerged. It is a sophisticated solution that integrates organoid development with the precision of microfluidic engineering. Since the start of the 21st century, advancements in microfluidics have enabled precise fluid flow control. This allows for the simulation of *in vivo* conditions, such as blood flow and interstitial shear stress. These innovative platforms integrate various mechanical components. These include micropumps, microvalves, and sensors. Their role is to accurately regulate dynamic parameters like flow rate and pressure gradients. In practice, these sensors are also used to verify and stabilize the imposed mechanical microenvironment (e.g., monitoring pressure/flow fluctuations and barrier status), thereby improving long-term reproducibility. Furthermore, elastomeric materials, such as polydimethylsiloxane (PDMS), are used. The incorporation of smart materials—like thermo- or photo-responsive hydrogels—is also a key. Together, they allow for the dynamic regulation of matrix stiffness and elasticity [7]. These systems are further combined with other technologies. Programmable actuators, high-frequency vibration platforms, and micropillar arrays are integrated. This combination enables the precise simulation of physiological ranges for cyclic tensile strain and mechanical gradients [8]. These synergistic technological innovations are transforming organoids. They are becoming high-fidelity micro-physiological systems. This transformation significantly enhances their utility in critical areas like disease research, drug screening, and regenerative medicine.

In conclusion, despite several reviews on organoids or OoC, a dedicated synthesis focusing on mechanobiological principles and their engineering implementation is still lacking. This review aims to bridge that gap. This synthesis would specifically focus on the mechanical aspects and their integrated control systems. Current reviews frequently overlook an important interplay. It is the intricate interplay between hardware precision and biological response. This work directly addresses that disparity. It offers a timely overview of two key areas. First, it reviews the applications of mechanical force. Second, it highlights the engineering innovations driving the next generation of physiologically relevant organ models.

The primary aim of this work is to provide a comprehensive overview. It focuses on the role of mechanical force in both organoid culture and organ-on-a-chip. This overview is based on

recent literature and is conceptually summarized in Fig. 1. First, the review revisits fundamental theories concerning the impact of mechanical stimuli on cell behavior. It examines how specific signals—such as pressure, fluid shear stress, adhesion forces, and contractile forces—activate cellular mechano-sensors. It also explores the different mechano-transduction pathways involved in this process. Subsequently, the review discusses the specific roles of mechanical factors. These roles are critical in organoid formation, maintenance, and functional expression. It examines the effects of different mechanical stimuli, including fluid shear stress, matrix stiffness, and tensile stress. The effects on organoid morphology, structure, and function are analyzed. The review also highlights recent advances concerning the role of mechanics in organ-on-a-chip. For example, it covers the use of microfluidic systems to simulate *in vivo* blood flow shear stress. Another example is the use of tunable matrix stiffness platforms to investigate matrix-cell interactions. Finally, the article discusses future prospects for leveraging mechanical control in this field. Potential applications span several areas, including disease modeling, drug screening, tissue engineering, and regenerative medicine. We anticipate that further exploration of the interplay between mechanics and organ-on-a-chip will open new avenues for biomedical research. This progress is expected to advance the development of precision medicine and personalized therapies.

2 The fundamentals of mechanical forces involving cells

Cells, as the fundamental units of life, are subject to numerous interacting forces in their environments. These forces include, but are not limited to, pressure, compression, shear stress, adhesion, and cell contractility. They act upon cells collectively, influencing cell shape, movement, division, and interactions with other cells. Therefore, cellular mechano-transduction is a key regulator of many biological processes. Recent quantitative studies in mechanobiology have demonstrated this regulation. They show that the magnitude and direction of physical cues can directly regulate cell fate decisions, morphogenesis, and tissue organization [22]. These cues typically range from pressures of several kilopascals to shear stresses of 0.1–10 pascals. Importantly, these mechanical forces act across multiple spatial scales. At the cellular scale, they affect the cytoskeleton and nucleus mechanics. At the tissue scale, they govern collective deformation, the extracellular matrix (ECM) remodeling, and intercellular force transmission [23].

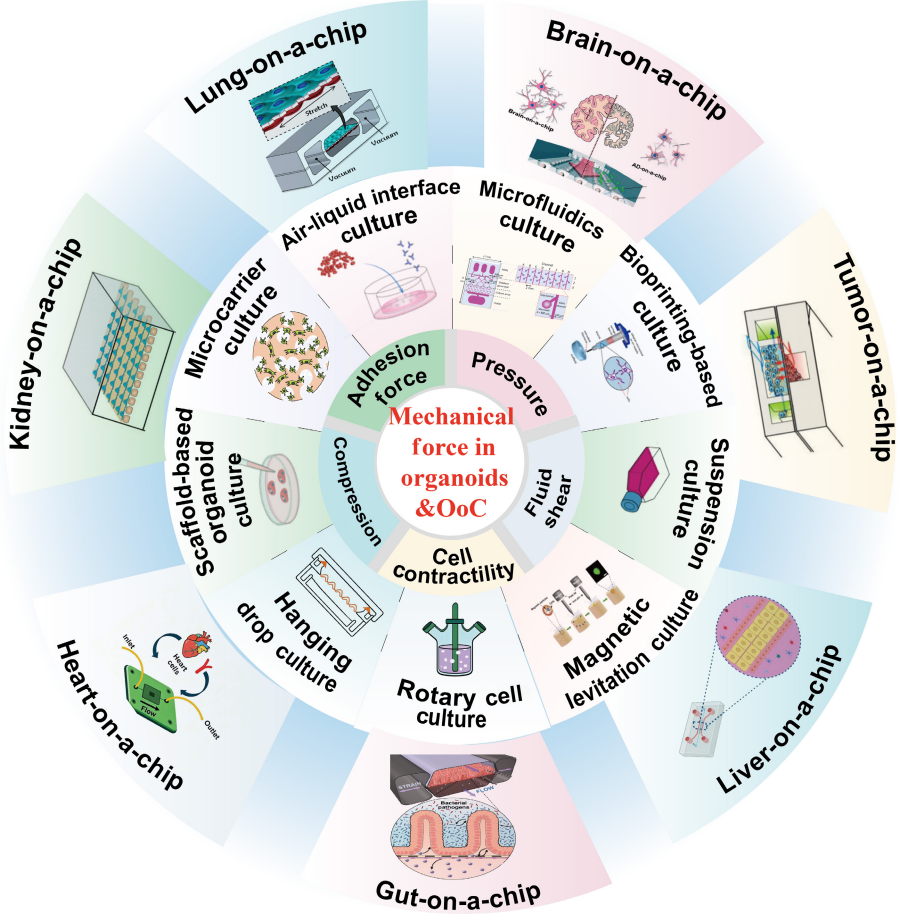

Figure 1 The role of mechanical force at cellular, organoid culture, and OoC levels. Reproduced with permission from Ref. [9], © Liu, J. et al. 2024. Reproduced with permission from Ref. [10], © Abuwatfa, W. H. et al. 2024. Reproduced with permission from Ref. [11], © Koh, B. et al. 2020. Reproduced with permission from Ref. [12], © Elsevier Inc. 2018. Reproduced with permission from Ref. [13], © WILEY-VCH Verlag GmbH & Co. KGaA, 2020. Reproduced with permission from Ref. [14], © Xi'an Jiaotong University 2018. Reproduced with permission from Ref. [15], © Shin, J. et al. 2022. Reproduced with permission from Ref. [16], © American Association for the Advancement of Science 2010. Reproduced with permission from Ref. [17], © Wang, D. et al. 2022. Reproduced with permission from Ref. [18], © Bein, A. et al. 2018. Reproduced with permission from Ref. [19], © Tian, C. T. et al. 2022. Reproduced with permission from Ref. [20], © Elsevier Ltd. 2022. Reproduced with permission from Ref. [21], © WILEY-VCH Verlag GmbH & Co. KGaA 2020. Some of the images are self-drawn by the author.

Understanding these principles provides a critical foundation. This foundation is essential for exploring the mechanobiology of organoids and organ-on-a-chip. Both systems depend on the integration of such mechanical forces across different scales.

2.1 Pressure and compression forces

In cell biology, pressure and compression are two distinct forms of mechanical force. They profoundly influence cell function through unique physical mechanisms. Pressure primarily manifests as a force acting uniformly on the cell surface or interior. This occurs within a fluid or static environment, as shown in Fig. 2(a). For instance, hydrostatic pressure is transmitted through fluids. Examples include the high-pressure environment for deep-sea organisms or blood pressure within vessels. This pressure directly acts on the cell membrane or organelle membranes. The result is changes in membrane tension [24]. Typical physiological hydrostatic pressures exist within specific ranges. In interstitial tissues, it ranges from 1000 to 5000 Pa. In blood vessels, the range is 12,000 to 16,000 Pa. Pathological hypertension, however, may exceed 20,000 Pa [25]. Such quantitative ranges are essential for a key comparison. They allow researchers to compare *in vivo* mechanical cues with those reproduced *in vitro*. In response to pressure, cells activate specific mechanisms. They use both passive structural buffering and active mechanical adaptation. The goal is to mitigate potential mechanical damage. For instance, proteins on the cell membrane can form funnel-like structures. Through their invaginative properties, these structures buffer changes in membrane tension. At higher pressure levels, cells can also achieve "nuclear decoupling". They accomplish this by inhibiting the expression of key LINC complex components. This action weakens the mechanical connection between the cytoskeleton and the nucleus. It thus protects the nucleus from excessive pressure.

In contrast, compression force refers specifically to the directional squeezing of cells or tissues. This force is generated by external mechanical loads. It is typically applied perpendicular to the cell surface or along a specific axis. For example, chondrocytes experience periodic compressive forces during joint movement. These forces are transmitted through the ECM to the cytoskeleton. This transmission ultimately induces cell deformation [26]. Compressive stress can also be generated within tumor tissues. This occurs due to spatial confinement. Such stress can inhibit angiogenesis and promote invasive behavior. The cellular response to compression depends on its magnitude. Moderate compression, typically between 1000 and 10,000 Pa, can promote beneficial

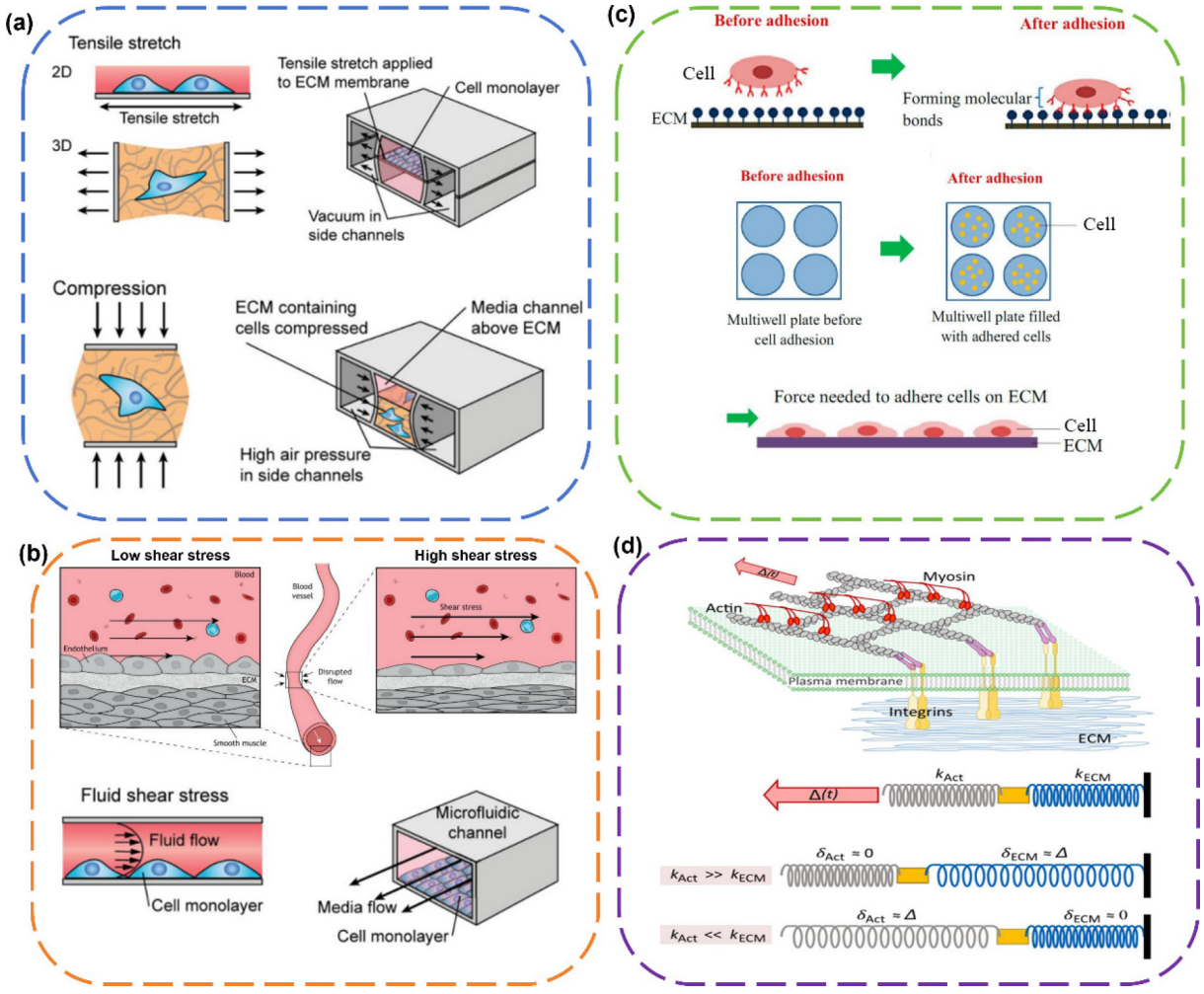

Figure 2 (a) Schematic illustration of tensile stretch and compression and examples of how these stimuli could be implemented in organ-on-chip systems. Reproduced with permission from Ref. [49], © Thompson, C. L. et al. 2020. (b) Schematic illustration of how shear stress can affect blood vessels. Reproduced with permission from Ref. [50], © The Company of Biologists Ltd. 2023. (c) Schematic diagram of cell adhesion attachment events. Reproduced with permission from Ref. [51], © Ahmad Khalili, A. et al. 2015. (d) A simple model of cellular contractility. Reproduced with permission from Ref. [47], © Feld, L. et al. 2020.

processes. These include cartilage ECM synthesis or osteogenic differentiation. However, excessive compression, often above 50,000 Pa, can induce cell apoptosis [27]. This mechano-transmission to the nucleus further regulates gene expression and cell fate. It does so through mechanosensitive transcription factors like Yes-associated protein (YAP)/Transcriptional coactivator with PDZ binding motif (TAZ). Recent studies highlight another key factor. The viscoelasticity of the ECM significantly modulates how compression propagates through tissues [28]. For instance, in viscoelastic hydrogels, slow stress relaxation helps dissipate compressive force. This reduces nuclear strain and helps protect genomic integrity[29]. Conversely, purely elastic matrices tend to amplify mechanical deformation. This effect is particularly crucial within organoid systems. Here, the internal lumen pressure, typically ranging from 500 to 2000 Pa, interacts with the stiffness of the surrounding matrix. These two factors jointly determine key morphological outcomes. They govern processes like cyst expansion and the emergence of branching structures [30].

In summary, pressure governs global volumetric regulation. Compression, on the other hand, induces local deformation and mechanoresponsive signaling. Together, they define a bidirectional feedback loop. This loop connects cell mechanics with microenvironment remodeling. However, despite extensive studies at the single-cell and tissue levels, a significant gap remains. Quantitative mapping of pressure and compression within 3D organoids is still limited. Currently, very few systems can independently tune both internal hydrostatic pressure and external matrix confinement. Future integration of microfluidic pressure control or deformable matrices into organoid-on-chip systems will enable a more precise study.

2.2 Fluid shear stress force

FSS is the tangential force generated by fluid flowing over a surface. This force arises due to velocity gradients within the fluid. Its magnitude is determined by three key factors: fluid viscosity, flow velocity gradient, and the contact area. In biological systems, FSS is a dominant mechanical cue. It regulates essential processes like cell polarity, morphology, and differentiation. The levels of shear stress vary significantly. In capillaries, it typically ranges from 0.1 to 2 Pa. In arteries, the range is higher, from 10 to 20 Pa. In tissue interstitial flow, it can be as low as 10^{-3} to 10^{-2} Pa [31]. These extremely low values are particularly relevant for organoid culture. Here, microfluidic shear can drive critical processes like cell polarization and vascularization [32].

In blood vessels, blood flow exerts shear stress on the endothelial surface of the vessel wall, as shown in Fig. 2(b). Vascular endothelial cells (ECs) play a critical role in regulating circulatory function. Shear stress modulates EC function through several actions. It activates specific mechano-sensors, triggers signaling pathways, and alters gene and protein expression [33]. The type of shear stress dictates the cellular response. Oscillatory shear stress (OSS), typically below 0.4 Pa, promotes a pro-inflammatory endothelial phenotype. In contrast, steady laminar shear stress (LSS), usually above 1 Pa, helps maintain an anti-inflammatory and quiescent state[31]. These distinct responses arise from specific signaling pathways. One key pathway involves Piezo1-mediated Ca^{2+} influx and the subsequent downstream modulation of YAP/TAZ [34]. The magnitude of fluid shear force can modulate Piezo1 ion channels on the cell membrane. It influences their distribution and activation threshold. This, in turn, affects the intensity of the calcium signaling they mediate. Notably, different force patterns produce different effects. Low but sustained shear may promote the localized aggregation and stabilization of Piezo1 channels. This maintains a mechanosensitive state. In contrast, shear force above a certain range may induce broader channel activation. It can also amplify downstream signaling pathways. This force-regulated control over Piezo1 directly determines key features of Ca^{2+} signaling. These features include its amplitude, oscillation frequency, and spatial extent. Ultimately, this process indirectly modulates cell behaviors. Affected behaviors include migration, inflammatory cytokine release, and changes in gene expression. Therefore, precise control of shear force magnitude is a critical strategy. It allows regulation of cell fate and behavior through Piezo1 channels.

It is well-established that atherosclerosis is a complex chronic inflammatory disease. It involves damage and repair processes in the arterial wall. Atherosclerotic plaques commonly form in areas with specific blood flow. These are curved or bifurcated arteries dominated by OSS. OSS can induce a pro-inflammatory phenotype in endothelial cells. This increases cellular inflammation, oxidative stress, mitochondrial dysfunction, and endothelial permeability. Together, these effects promote atherosclerosis progression [35]. Conversely, straight arterial segments experience stable LSS. LSS promotes an anti-inflammatory phenotype in endothelial cells. It also improves endothelial function. Consequently, it inhibits atherosclerosis progression [36].

Beyond its protective effects on endothelial cells, laminar shear stress also influences endothelial metabolism. It can alter gene expression, leading to changes in endothelial phenotype and vascular homeostasis. Translating vascular FSS to 3D organoid and organ-on-chip systems is challenging. Excessively high shear can disrupt fragile epithelia. Conversely, insufficient flow limits nutrient diffusion. Controlled laminar shear in the range of 10^{-3} to 10^{-2} Pa is optimal. It optimizes endothelial network formation and morphogen distribution [37]. Thus, accurately reproducing physiological shear regimes is essential. It is key to achieving functional vascularization in engineered organoids.

Although modern microfluidic platforms allow precise modulation of flow, challenges remain. A major challenge is dynamically tuning shear gradients within complex 3D cultures. Most current studies still rely on simplified parallel-channel geometries. These fail to capture the spatial heterogeneity of native tissues. Future organoid-on-chip systems will need to integrate new capabilities. These include real-time shear sensors and adaptive flow control. Such integration will allow feedback-driven adjustment of local FSS. This will enable the quantitative dissection of mechanosensitive pathways.

2.3 Adhesion force

Adhesion force describes the interaction force that enables one material to attach to another. At the cellular level, these forces arise from specific receptor-ligand interactions. These interactions connect cells to each other or to the ECM. Focal adhesions (FAs) are key structures in this process. These complexes transmit traction stresses, typically ranging from 1000 to 5000 Pa [38]. Such mechanical linkages allow cells to dynamically sense and remodel their immediate microenvironment. Cell adhesion forces originate from multiple sources. These include cell-to-cell interactions, cell-to-ECM interactions, and forces involving internal cellular structures, as illustrated in [39]. This adhesion is primarily mediated by specific

cell adhesion molecules (CAMs) present on the cell surface. The process is critical for many physiological functions. Key examples include embryonic development, cell migration, tissue remodeling, and maintaining homeostasis. Furthermore, it acts as a vital mode of communication between cells. In multicellular organisms, every cell must interact with its neighbors and environment. These interactions are largely accomplished through cell adhesion. Adhesion forces enable cells to firmly attach to the ECM. This attachment is vital for cell stability and survival. In fact, losing contact with the ECM can trigger a specific type of programmed cell death, known as anoikis [40]. Cells navigate a complex extracellular environment filled with chemical and mechanical signals. They rely on adhesion receptors to probe and interpret this microenvironment. Focal adhesions serve as crucial multiprotein complexes. They link the adhesion receptors to the cell's internal actin cytoskeleton. The forces transmitted through these complexes can significantly remodel the ECM. This plays important roles in both healthy processes, like wound healing, and diseases, such as cancer metastasis.

Changes in cell adhesion forces can be critical in various diseases. These include arthritis, cancer, osteoporosis, and atherosclerosis [41, 42]. In human cancers, cell adhesiveness is often notably reduced. This decrease allows cancer cells to disobey normal tissue organization. Consequently, it leads to the disruption of tissue structures, a hallmark of malignant tumors. Tumor cells exhibit altered adhesion to the ECM. This alteration can correlate with their potential to invade and spread to other parts of the body. Adhesion forces also influence stem cell differentiation. They provide specific cues that guide stem cells toward particular cell lineages. Research shows that adhesion to different types of ECM affects the pathways of stem cell differentiation [43]. In the vascular system, these forces help maintain the integrity of the endothelial cell layer. This is essential for preventing the leakage of blood components.

In organoid and organ-on-chip systems, adhesion mechanics play a central organizing role. ECM composition and integrin patterning guide organoid polarity. Dynamic control of adhesion strength can direct tissue fusion or separation during development. Despite progress, most current organoid models rely on static ECMs like Matrigel. They offer limited tunability. Next-generation systems are emerging. They may use synthetic matrices with programmable adhesion sites or light-responsive integrins [44]. Such advances will allow real-time modulation of cell-matrix binding. This capability will enable deeper study of how adhesion drives self-organization and morphogenesis in laboratory settings.

2.4 Cell contractility force

Cell contractility denotes the ability of cells to generate contractile motion and forces. This property can be broadly divided into active and passive components. Active contractility is an intrinsic property of the actomyosin cytoskeleton. It does not require external stimulation. Instead, it emerges from myosin-driven sliding of actin filaments. This sliding action produces a well-defined, time-dependent contractile displacement, noted as $\Delta(t)$, within the cell. At the whole-cell scale, these intrinsic displacements give rise to significant forces. Contractile forces are typically on the order of 100 to 1000 nN. Cortical stresses range from 1000 to 10,000 Pa. These internal forces are balanced by cell-matrix adhesion and the stiffness of the ECM. This balance helps maintain mechanical homeostasis within the cell [45, 46].

A minimal mechanical model, proposed by Feld et al., effectively represents this process. The model features an actomyosin "contractile unit" connected in series with an ECM spring. In this framework, myosin motors prescribe the intrinsic displacement $\Delta(t)$. Integrin-based adhesions then transmit this displacement to deform the surrounding matrix. A key insight is that the measured contractile force scales linearly with ECM rigidity. Importantly, the underlying displacement $\Delta(t)$ itself is independent of the matrix's rigidity. In this context, active contractility refers to the cell-intrinsic program $\Delta(t)$. The role of adhesion forces and ECM mechanics is to determine how this preset displacement is converted into observable forces at the cell-ECM interface. This relationship is illustrated in [47].

In contrast, passive contractility describes contractile responses that are evoked by external cues. These cues can include mechanical loading, soluble biochemical factors, or temperature changes. Such stimuli activate intracellular signaling pathways, which in turn modulate actomyosin activity and cytoskeletal organization. In both active and passive cases, stress fibers and cortical actomyosin networks serve as the primary force-generating structures. Their contractility has a wide-ranging influence. It affects fundamental cellular processes like cell migration, mechanosensing, and shape changes. At a higher level, it also governs tissue-level events such as morphogenesis, collective cell movement, and the self-organization of organoids.

Some cells generate contractile forces using specialized structures called stress fibers. These fibers are composed of actin and myosin filaments. This contractility is crucial for basic cellular functions, including migration and mechanosensing. Furthermore, muscle cell contraction is fundamental to organismal movement. Skeletal muscle contraction enables body movement and respiration. Cardiac muscle contraction powers blood circulation. Smooth muscle contraction facilitates actions like peristalsis in the digestive tract. Together, these contractile activities are vital for normal physiological function. Contractile forces also influence intracellular transport, nuclear deformation, and gene expression. In stem cells, the level of cytoskeletal tension can bias differentiation fate. High tension often promotes osteogenic lineages, while low tension favors neurogenic fates. At the tissue level, coordinated contractility drives key morphogenetic events. These events include apical constriction, lumen formation, and collective cell migration.

In organoid and organ-on-chip systems, contractility acts as the primary mechanical engine. It drives morphogenesis and tissue motion. Researchers can precisely control this process by modulating contractile signaling. For example, they can use Rho-associated protein kinase (ROCK) inhibition or optogenetic myosin activation. This control allows for the shaping of organoid geometry and can induce rhythmic contractions in heart- or gut-on-chip models. However, a current limitation is that most models treat contractility as a passive outcome. It is not yet widely used as an active design parameter. Future work aims to integrate dynamic feedback control of actomyosin tension with real-time imaging. This advancement will enable the programmable mechanical regulation of organoid development. Ultimately, it will help bridge the gap between cellular biomechanics and tissue-level morphogenesis.

Altogether, integrating these specific mechanical forces provides an unified framework. These forces include pressure, compression, shear, adhesion, and contractility. As shown in Table 1, this work summarizes the roles of these forces in cells. Their integration is crucial for understanding organoid mechanobiology. Researchers can now achieve this by coupling quantitative force control with organoid-on-chip technology. This powerful combination allows

Table 1 Role of mechanical forces in cells

Mechanism	Source/definition	Core mechanism	Primary function	Typical applications	References
Pressure	Fluid transmission Solute gradient	Alters membrane tension Activates mechanosensitive channels Triggers UPR in ER stress	Maintains osmotic homeostasis Regulates cell volume	Physiological: deep-sea adaptation Pathological: hypertension, cell lysis/crenation	[24]
Compression	Directional external load	Cell deformation Nuclear mechanosensing Modulates YAP/TAZ pathway	Controls proliferation/differentiation Enables mechanoadaptation	Physiological: chondrocyte ECM synthesis, MSC osteogenesis Pathological: tumor EMT/chemoresistance, apoptosis	[26, 48]
Fluid shear stress	Tangential fluid force	Mechano-sensors Activates PKC/cAMP/Ca^{2+} pathways Alters gene expression	Maintains endothelial barrier Regulates inflammation/metabolism	Protective: LSS, anti-atherogenic Pathological: OSS, pro-atherogenic	[33]
Adhesion force	Cell–cell/ECM binding	CAM-mediated cadherins/integrins Focal adhesions link ECM: actin cytoskeleton	Prevents anoikis Guides migration/differentiation	Physiological: embryogenesis, vascular integrity Pathological: cancer metastasis, arthritis	[39]
Cell contractility	Actin-myosin sliding/stimuli-induced	Myosin motor activity Stress fiber formation Signaling pathway activation	Drives cell migration Powers muscle function Facilitates ECM remodeling	Essential: organ movement, circulation Cellular: molecular transport, fate determination	[47]

them to dissect a key process. They can examine how physical microenvironments guide critical biological events.

3 Mechanical force in organoid culture

Organoids are miniature, organ-like structures cultivated *in vitro*. They are typically generated using stem cells or specific tissue cells through 3D culture techniques. These techniques aim to simulate key developmental processes that occur *in vivo*. As a result, organoids can replicate the architecture and certain functions of real organs. This makes them a powerful tool for various medical research applications. However, a critical challenge lies in enabling these cells to self-organize into complex 3D structures within a laboratory dish. Success in this endeavor depends critically on creating an appropriate growth environment. Different cultivation methods can produce significantly different effects, particularly regarding the mechanical forces experienced by the cells and their subsequent behavior. These factors are fundamental, as they ultimately determine the growth patterns and functional performance of the resulting organoids.

3.1 Solid substrates-based organoid culture methods

Microcarrier culture, air-liquid interface (ALI) culture, microfluidic chip methods, and bioprinting can all be viewed as developments derived from traditional scaffold-based methods. They extend the core concept of the scaffold approach to some extent. This concept involves enhancing cell growth and functional maintenance by providing physical support. However, each of these methods also possesses unique advantages and distinct technical characteristics. This group of methods primarily relies on physical support structures or external control. Their goal is to provide a 3D culture environment for cells. In practice, cells are typically attached to or embedded within scaffolds or carriers. Alternatively, they may be precisely regulated within microfluidic systems. These methods share a common emphasis. They aim to promote cell growth and differentiation by offering specific conditions. These conditions include physical support, controlled fluid flow, or tailored surface properties.

3.1.1 *Scaffold-based organoid culture methods*

Scaffold-based culture is a technique that uses solid scaffolds to provide three-dimensional support for cells. This method aims to promote cell growth, differentiation, and tissue development by mimicking the *in vivo* microenvironment. It optimizes cell behavior and encourages organoid formation through physical and biochemical signals from the scaffold. Several common methods exist for scaffold-based cultures. In the Dome Method, cells are mixed with thermo-responsive hydrogels and pipetted as droplets into culture vessels. Media is then carefully layered over the dome-shaped hydrogel to support spheroid formation. With Insert Wells, hydrogel-cell mixtures are placed into porous inserts that are suspended in wells filled with media. Spheroids form at the insert bottom, allowing nutrient exchange through the hydrogel matrix. In gel-bottom support, a thick layer of hydrogel covers the bottom of a well, and the cell suspension is placed on top. Finally, in the embedding method, cells are uniformly suspended in hydrogel, poured into vessels, and covered with media, as illustrated in Fig. 3(a) [10].

The physical properties of the scaffold directly influence the mechanical forces applied to the cells. These properties significantly affect cell morphology and function. For example, the stiffness of the scaffold influences cell shape and motility. Meanwhile, the porosity of the scaffold affects the supply of nutrients and oxygen to the cells. The micro-architecture of the scaffold also provides important physical cues. For instance, in an electrospun scaffold, the alignment of nanofibers can guide cell orientation. This phenomenon, known as contact guidance, exerts topographic forces on cells. When cells adhere to parallel-aligned nanofibers, the anisotropic physical cues generate cytoskeletal tension. This tension directs cell migration along the axis of the fibers. In addition to the scaffold's inherent properties, these culture systems involve interactions with other mechanical and biochemical forces. These mechanical forces primarily regulate cell processes like proliferation, differentiation, and migration. The regulation occurs through interactions between integrins and the cytoskeleton. Integrins connect with the cytoskeleton, activating downstream signaling

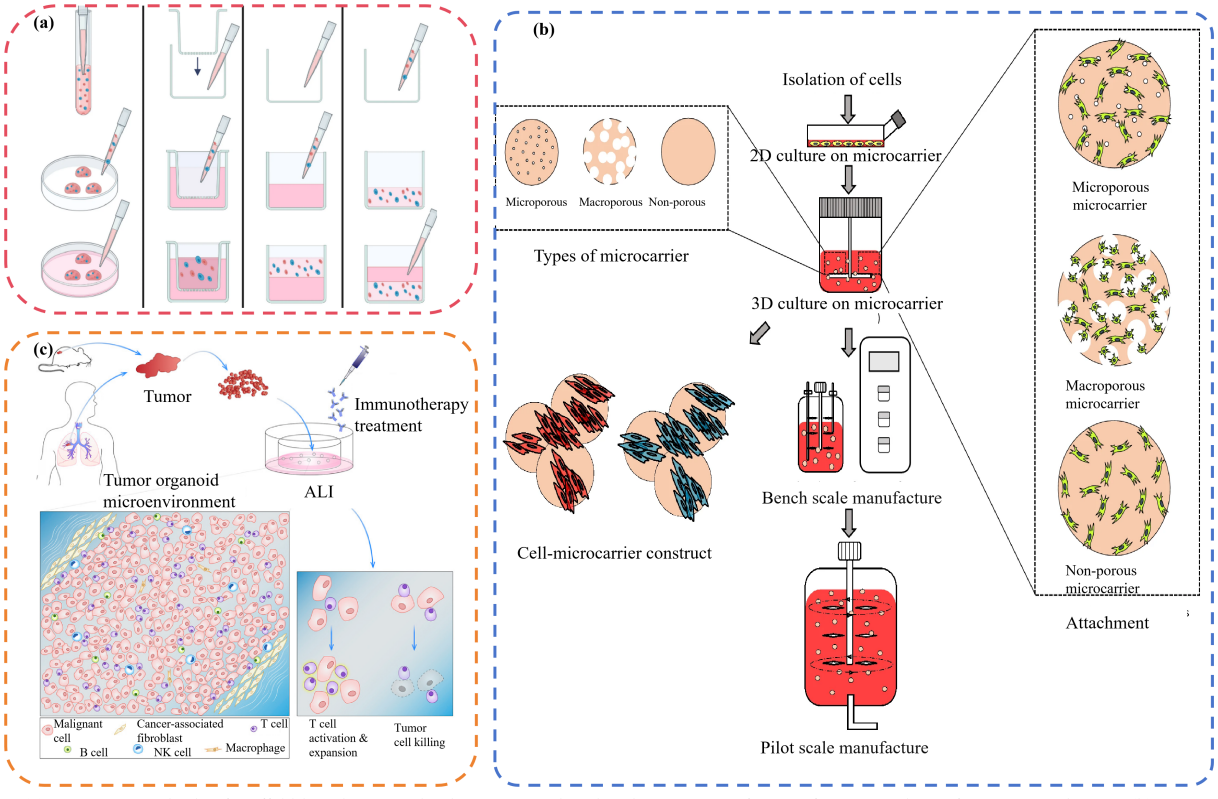

Figure 3 (a) Common methods of scaffold-based organoid cultures. Reproduced with permission from Ref. [10], © Abuwatfa, W. H. et al. 2024. (b) Major types of microcarrier. Reproduced with permission from Ref. [11], © Koh, B. et al. 2020. (c) Schematic diagram of the experimental procedure for tumor organoid-T cell co-culture, illustrating the 3D architecture of the matrigel scaffold. Reproduced with permission from Ref. [12], © Elsevier Inc. 2018.

pathways such as the Rho GTPase pathway. This activation ultimately influences cell morphology and function. Furthermore, external dynamic stimulation can be applied. For example, in a flexible elastomer-based scaffold, cyclic stretching causes the scaffold to deform. This mechanical strain is then transferred to the adhered cells as tensile stress. The stress activates mechanosensitive ion channels and influences cell alignment and ECM remodeling.

3.1.2 *Microcarrier culture methods*

The core difference between scaffold-based culture and microcarrier culture lies in how cells bind to the support structure. Scaffold-based methods simulate the *in vivo* microenvironment by providing a fixed three-dimensional scaffold. This promotes cell growth and differentiation. However, the method has several limitations. These are tied to scaffold design, material selection, and the characteristics of cell-scaffold interactions. A specific challenge arises in high-density cell culture. In this scenario, the pore structure and support strength of the scaffold may become insufficient [13]. In contrast, microcarrier culture utilizes small carrier particles to provide support for cells. This approach enables more efficient high-density culture. It also facilitates large-scale cell expansion. Microcarriers typically have a large specific surface area. This characteristic helps enhance cell adhesion and improves uniformity during the culture process.

The process of cell attachment to microcarriers involves two main phases. First is an initial adsorption phase. This phase is primarily driven by electrostatic interactions and van der Waals forces. These forces act between the negatively charged cell membrane and the microcarrier's surface charge. This is followed by a strengthening phase. This later phase is mediated by cellular adhesion molecules. It leads to the development of firm adhesion plaques and eventual cell spreading. During microcarrier culture, fluid shear stress is generated by agitation. This stress acts on both the microcarriers and the cells. It regulates mechanoreceptors on the cell membrane, thereby influencing cell proliferation and differentiation. Additionally, centrifugal forces are present in rotating or shaking culture systems. These forces regulate cell behavior by influencing how cells adhere to and interact with the microcarriers. Specifically, centrifugal force can help enhance cell attachment. This improves the overall efficiency of organoid formation. There are three major types of microcarriers: non-porous, microporous, and macro-porous. Cells attach differently based on the porosity of the microcarrier. In general, cells attach to the surface of non-porous or microporous carriers. Microporous carriers, however, provide larger internal spaces. These spaces allow cells to attach within the inner part of the microcarrier, as illustrated in Fig. 3(b) [11].

3.1.3 *Air–liquid interface culture methods*

Compared to microcarrier culture, ALI culture simulates natural conditions in an *in vitro* environment. It uses the interface between gas and liquid phases as a support platform for cell growth. This method can better maintain the three-dimensional growth state of cells. It is particularly suitable for cell types that require gas exchange, such as epithelial cells [52]. Air–liquid interface culture cultivates cells at the interface between gas and liquid. It utilizes the gas permeability of the interface to provide oxygen and nutrients. The process of force generation at the air–liquid interface begins with two main types of forces. These are the cohesive forces between liquid molecules and the adhesive forces between the

liquid and the solid substrate. Molecules at the liquid–air interface experience a net inward pull. This pull is due to unbalanced cohesive forces, which creates surface tension (γ). Surface tension is quantified as force per unit length (N/m). This interfacial tension minimizes the surface area of the liquid film. The result is a stable, thin layer atop which cells can adhere and grow.

During ALI culture, the primary forces are surface tension and capillary forces. Capillary forces arise within the porous structure of the membrane or a semi-solid matrix. When the culture medium partially wets the membrane pores, capillary action draws liquid into the pores. This action creates a concave meniscus, a process that occurs when the contact angle is less than 90°. According to the Young–Laplace equation ($\Delta P = \gamma(1/R_1 + 1/R_2)$), this curvature generates a negative Laplace pressure. This pressure exerts a compressive force on the cells attached to the membrane. The magnitude of this force is inversely proportional to the pore radius. This means that finer pores generate stronger capillary pressures. These forces play a key role in regulating cell distribution on the interface. They influence cell adhesion, growth, arrangement, and morphology. They also affect how cells interact with the supporting matrix, thereby promoting normal cell function. Additionally, advanced ALI systems can be integrated with microfluidics. In these systems, perfusion of basolateral media generates subtle fluid shear stress across the porous membrane. This flow-induced stress is significantly lower than in microcarrier cultures, typically on the order of 10^{-3} to 10^{-2} Pa. These forces effectively simulate the *in vivo* tissue interface environment. They provide more natural growth conditions for cells.

By regulating ALI forces and oxygen concentration gradients, this method can maintain the structure and function of organoids long-term. This approach simulates the authentic *in vivo* environment. For example, tumor tissue samples have been obtained from patients and mice. The tumor organoid microenvironment containing multiple cell types was constructed using ALI culture technology. This technology highly simulates the complex heterogeneity of tumors *in vivo*, as illustrated in Fig. 3(c) [13]. The ALI-cultured patient-derived tumor organoids preserve the native tumor immune microenvironment. Researchers can study tumor-immune interactions and immunotherapeutic responses. Beyond biochemical cues, this system incorporates important biomechanical forces. Examples include fluid shear stress and matrix stiffness. These forces influence critical immune cell behaviors. Affected behaviors include infiltration, activation, and cytokine secretion. These mechanical factors can be evaluated alongside pharmacological interventions. Evaluation is often conducted using techniques like flow cytometry. This method tracks immune subset dynamics and the upregulation of effector molecules. This approach effectively links the mechanobiological context to functional immunoreactivity. Furthermore, the ALI platform supports *ex vivo* drug testing. It does this by replicating biomechanical niches found *in vivo*. This capability helps identify patient subgroups. These subgroups are likely to respond to specific therapies, such as adoptive T-cell therapies or combination regimens. Thus, the ALI-cultured organoid model serves a specific function. It acts as a biomechanically competent "avatar". This avatar is used for optimizing personalized immunotherapy.

3.1.4 *Microfluidics culture methods*

ALI systems primarily rely on semi-static conditions, lacking precise control over fluid shear stress and pressure gradients, which are critical for simulating physiological processes such as blood flow or interstitial fluid dynamics. This restricts their ability to model mechanosensitive cellular responses, such as endothelial shear sensing or tubular fluid transport in organ-specific contexts. To address these limitations, microfluidic chip methods enable fine spatiotemporal regulation of the cell growth environment by precisely controlling liquid flow within microscale channels. This approach allows for precise manipulation at the single-cell level. It also facilitates high-throughput analysis of various physical and chemical factors inside the microenvironment. For example, perfusion-driven shear stress in microfluidic chips activates mechanosensitive ion channels and promotes endothelial polarization, as illustrated in Fig. 4(a), where channel geometry dictates flow profiles to simulate physiological force distributions.

These methods are widely used for organoid culture and functional testing because they can simulate the *in vivo* fluid environment. The geometry of the microchannels is a critical design parameter. It directly impacts fluid dynamics and, as a result, influences cell behavior. In microfluidic chips, fluid flow primarily exerts mechanical effects on cells through two main mechanisms: shear stress and pressure gradients. As shown in Fig. 4(a), in a brain cancer chip model, the flowing culture medium applies shear stress to the cells. Simultaneously, pressure variations within the channels create pressure gradients [14]. The flow in microchannels is predominantly laminar, characterized by a very low Reynolds number ($Re << 1$). This means fluids flow in parallel streams without turbulence. This laminar flow regime enables the generation of stable, parallel streams containing different solutes within a single channel. The mixing of these solutes occurs primarily through diffusion at the interface between the streams. This process allows for the creation of precise and stable chemical gradients across the channel. Shear stress regulates key cellular processes like proliferation, differentiation, and function. It does this by acting on mechanoreceptors located on the cell membrane. For instance, fluid shear stress can simulate *in vivo* blood flow conditions. This simulation influences the alignment and function of endothelial cells [53]. The magnitude of the shear stress (τ) experienced by cells adhered to the channel surface can be estimated using fluid dynamics principles. Its value is a function of the flow rate, the channel geometry, and the fluid viscosity. Furthermore, microfluidic chip methods are extensively used for culturing vascular organoids. This technique can more realistically reflect the intravascular environment. It therefore provides an effective model for research on vascular-related diseases. Pressure gradients represent another key mechanical factor. They influence cell behavior by affecting mechanosensitive channels within cells. This effect alters intracellular ion concentrations and subsequent signal transduction. These pressure gradients can be generated by external pumps or by the specific design of the microfluidic network itself. They drive fluid motion according to the balance between pressure forces and viscous forces, as described by the Navier–Stokes equation for incompressible flow in the low Reynolds number regime. By finely regulating these mechanical factors, microfluidic chip methods enable detailed study of cellular responses to physiological environments at the microscale. An advanced application of this precise control is the generation of complex, overlapping gradients of soluble compounds inside the chip.

3.1.5 *Bioprinting-based culture methods*

Although microfluidic culture enables dynamic regulation of the

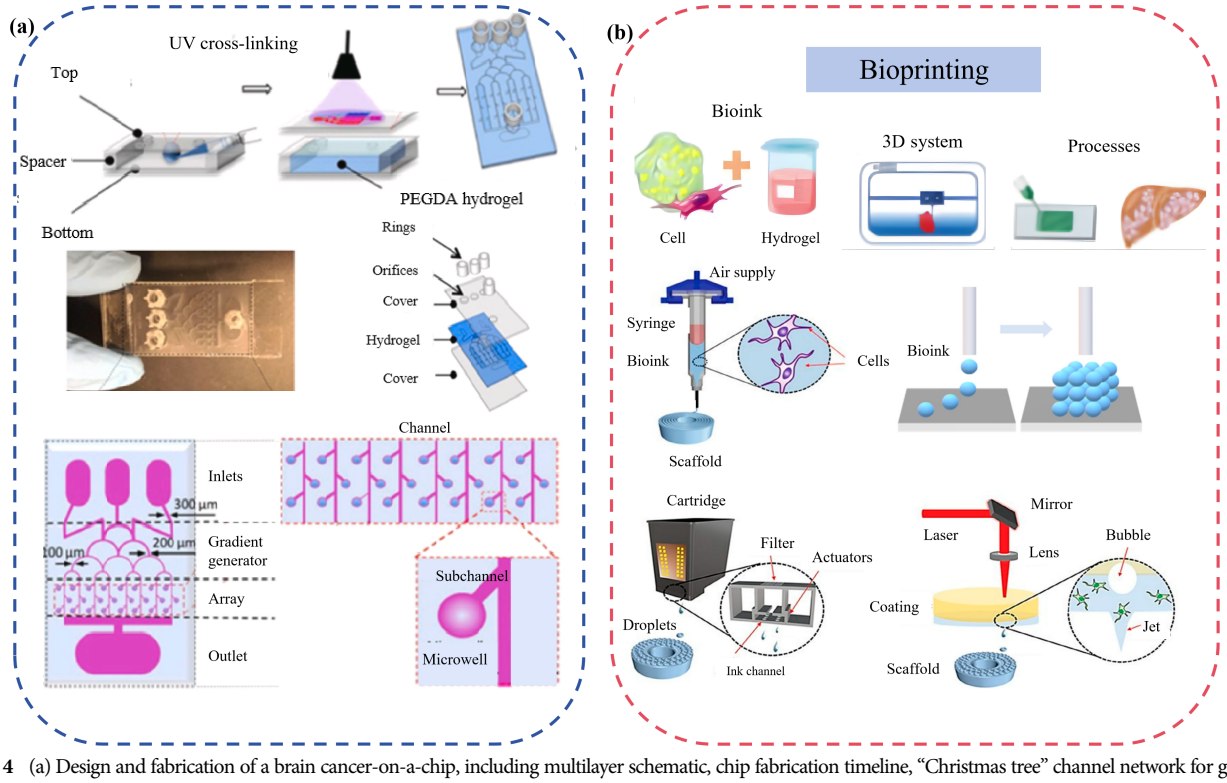

Figure 4 (a) Design and fabrication of a brain cancer-on-a-chip, including multilayer schematic, chip fabrication timeline, "Christmas tree" channel network for gradient generation, and final hydrogel device with microchannels and microwells. Reproduced with permission from Ref. [14], © Xi'an Jiaotong University 2018. (b) Process of bioprinting. Reproduced with permission from Ref. [15], © Shin, J. et al. 2022.

cellular microenvironment through precise control of hydrodynamic parameters within microchannels, they exhibit limitations in the spatial construction capability of 3D structures. Microfluidic systems typically rely on predefined channel geometries, making it difficult to freely construct multicellular tissue architectures with complex morphologies and heterogeneity, and their ability to simulate tissue-level mechanical properties remains constrained. To overcome these limitations, bioprinting technology has emerged, which achieves precise spatial control over 3D tissue structures through the layer-by-layer deposition of cell-laden bioinks and matrix materials. As shown in Fig. 4(b), the process begins by loading a bioink containing cells into a syringe. This bioink is then extruded through a nozzle by applying a shear force, ultimately creating 3D structures [15]. Bioprinting not only compensates for the shortcomings of microfluidics in constructing complex architectures but also enables more faithful simulation of the native tissue's mechanical microenvironment and cellular spatial organization via the cooperative printing of multiple materials and cell types, thereby significantly enhancing the morphological fidelity and functional maturation of organoids.

Several primary bioprinting techniques exist, including extrusion-based, inkjet, and laser-assisted bioprinting. Each of these methods generates distinct mechanical forces during the printing process. In extrusion-based bioprinting, a pneumatic or piston-driven system applies extrusion pressure. This pressure pushes the bioink through a nozzle. The flow rate (Q) during this process is governed by several factors. These factors include the nozzle radius (R), the pressure difference (ΔP), the bioink viscosity (μ), and the nozzle length (L). Their relationship follows a simplified relation: $Q \propto (\pi R^4 \Delta P)/(8\ \mu L)$. This extrusion process subjects the cells within the bioink to significant shear stress (τ). The magnitude of this stress is inversely proportional to the nozzle diameter. It is also directly proportional to the extrusion pressure and the bioink viscosity. It is important to note that excessive shear stress can compromise cell viability. Therefore, optimizing the bioink rheological properties and the printing parameters is necessary. Inkjet bioprinting operates on a different principle. It utilizes thermal or piezoelectric actuators to generate precise acoustic forces or pressure pulses. These forces eject tiny bioink droplets onto the substrate. In contrast, laser-assisted bioprinting employs a laser pulse. This pulse creates a high-pressure bubble that propels bioink onto a substrate. A key advantage of this method is that it exerts minimal mechanical stress on the cells. The mechanical forces involved in these processes play a crucial role. Through physical extrusion and deposition, they influence the arrangement, morphology, and distribution of cells within the printed structure. For instance, extrusion stress can regulate the spatial distribution and morphology of cells. This regulation ultimately affects the structure and function of the resulting organoids. Similarly, interfacial forces are important. They regulate the interactions between cells and the matrix. This regulation promotes cell adhesion and migration, thereby supporting the functional maintenance of organoids. The rheological properties of the bioink itself are critical for success. Properties such as viscosity and shear-thinning behavior are particularly important. Shear-thinning refers to the phenomenon where the bioink's viscosity decreases under shear stress during extrusion and recovers afterward. This behavior is essential for maintaining the structure's shape fidelity after printing while also protecting the cells from damage. Researchers are also developing advanced strategies to create more complex tissues. One such strategy is coaxial printing. This technique uses a nozzle-within-a-nozzle design to create core–shell filaments. It allows for the

simultaneous extrusion of multiple materials. For example, a core bioink containing parenchymal cells can be surrounded by a sacrificial shell material. After printing, the shell material can be removed. This removal creates perfusable channels that mimic native vascular networks. The process, however, requires complex interfacial tension control between the different bioinks to maintain a stable coaxial flow. The development of bioprinting technologies, along with other methods like scaffold-based culture and microfluidics, is built upon the foundational concept of providing cell support. While they provide support, these methods achieve a dynamic regulation of the cellular microenvironment through increasingly finer mechanical control. Mechanical factors are now understood to play a crucial role in cell culture. Key factors include shear stress, pressure gradients, interfacial forces, and adhesion forces. These forces determine cell growth, arrangement, differentiation, and functional maintenance. With continued technological advancements, future cell culture methods will focus even more on the precise control of the mechanical environment. The goal is to simulate *in vivo* physiological states with greater refinement and efficiency. This progress is expected to significantly advance fields like cell biology, tissue engineering, and regenerative medicine. Despite its advantages in precise spatial control and complex structure fabrication, bioprinting-based culture methods face several limitations that warrant consideration. Key challenges include the potential compromise of cell viability due to high shear stress during extrusion, particularly in nozzle-based systems, which can reduce post-printing cell survival and functionality. Additionally, the resolution of current bioprinting technologies remains constrained, often failing to replicate the nanoscale architectural details of native tissues, such as capillary networks. The reliance on bioinks also introduces variability, as their rheological properties must balance printability with biocompatibility, yet few materials fully mimic the dynamic mechanical properties of natural extracellular matrices [54].

The solid substrates-based approaches provide essential physical support and precise microenvironments for organoid growth through solid substrates, such as scaffolds, microcarriers, or bioprinted matrices. These methods excel in promoting cell adhesion, spatial organization, and mechanobiological cues like substrate stiffness. However, they often face limitations in simulating dynamic physiological conditions, such as insufficient nutrient diffusion, high variability, and restricted controllability under static cultures. However, the culture method without substrate can effectively compensate for this deficiency. The subsequent section introduces no substrates-based organoid culture methods, which leverage contactless dynamic forces to enable uniform cell distribution, enhanced metabolic exchange, and reduced mechanical stress.

3.2 No substrates-based organoid culture methods

No substrates-based organoid culture methods enable 3D cell growth and tissue formation without relying on solid scaffolds or fixed physical supports. Key examples in this group include magnetic levitation culture, spinner flask methods, rotary cell culture, and suspension drop methods. Instead of utilizing scaffolds, microcarriers, or printed matrices, these techniques leverage dynamic, physical forces, such as fluid motion, magnetic fields, or interface tension, to suspend, aggregate, and organize cells into complex tissue. A key advantage of these methods is their ability to provide a more homogeneous mechanical microenvironment, reduce shear-induced damage compared to some scaffold-based systems, and enhance nutrient exchange through continuous motion or levitation.

3.2.1 *Suspension culture methods*

Suspension culture achieves scaffold-free cell cultivation by using special suspension culture plates or vessels. Each well in these plates contains suspended cell clumps that self-assemble in the liquid environment to form three-dimensional structures. The design of these vessels is crucial, as it helps maintain the cells in a suspended state while still providing the necessary support for their growth and development. This specialized design is combined with constant orbital shaking on a platform shaker. The shaking motion generates a swirling flow within each well, creating a dynamic equilibrium. In this state, the sedimentation velocity of the cell clumps, which is governed by Stokes' law and is proportional to the square of the aggregate's radius, is balanced by upward convective currents in the fluid. This balance effectively keeps the cell aggregates in sustained suspension, preventing them from settling. The primary mechanical factors at play in this system are gravity and intercellular forces. Gravity influences how cells are distributed within the liquid, thereby regulating their aggregation and the self-assembly processes. Without constant agitation, gravity would simply cause the cells and small aggregates to settle at the bottom of the well. This settling concentrates the cells at the lowest point, increasing the frequency of cell-to-cell collisions. The orbital shaking motion periodically resuspends these settling aggregates. However, during the relatively quiescent phases between shaking cycles, gravity-driven sedimentation is still essential. It promotes the close cell-to-cell contact necessary for stable adhesion bonds to form. These adhesive interactions are primarily mediated by intercellular forces, which are facilitated by cell adhesion molecules such as cadherins. These forces regulate the interactions between cells and are crucial for promoting the formation of complex 3D structures. Under the combined action of gravity and these intercellular forces, suspension culture can effectively mimic key aspects of the *in vivo* microenvironment, which supports more normal cell growth and function. Researchers like Yohei Hayashi have observed specific cellular responses in this environment. For example, experiments have found that human induced pluripotent stem cells (hiPSCs) cultured in suspension are prone to spontaneous differentiation. Western blot analysis provided evidence for this, showing that the protein expression of markers like Pax6 and SOX17 was significantly increased under suspension conditions, as illustrated in Fig. 5(a) [55].

This suspension culture method has been successfully applied to cultivate various organoids, including liver organoids. By carefully optimizing parameters such as initial cell density and overall culture conditions, researchers can effectively simulate hepatocyte growth and function. This provides a reliable *in vitro* model for disease research. A critical aspect of process optimization involves balancing the mechanical forces. For instance, the revolutions per minute (RPM) is a key parameter. It controls the hydrodynamic shear stress and the mixing efficiency within the culture vessel. If the RPM is too low, it leads to inadequate mixing. This can cause aggregate sedimentation and result in hypoxia for the cells. Conversely, if the RPM is too high, the aggregates are subjected to damaging levels of shear stress. Similarly, the initial seeding density is another crucial factor. It determines the average distance between individual cells, which directly influences the probability of

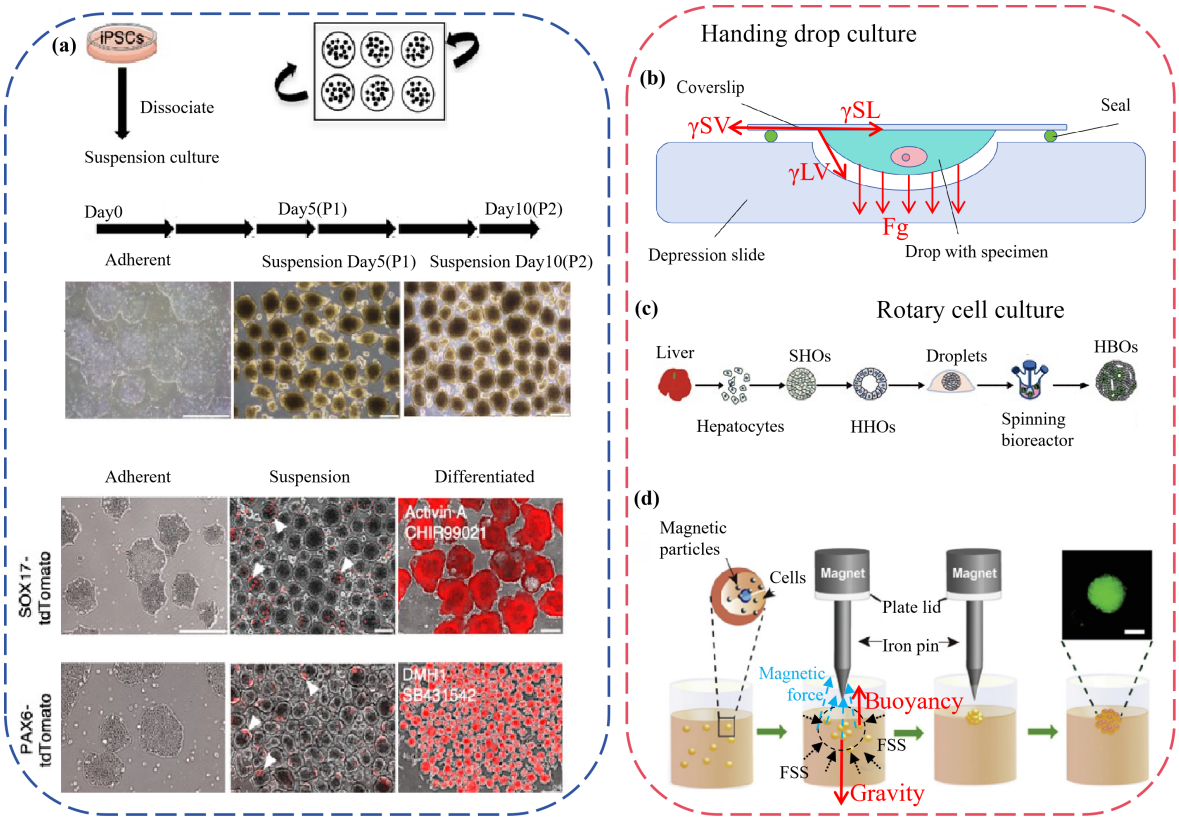

Figure 5 (a) Schematics representing hiPSCs in suspension conditions. Reproduced with permission from Ref. [55], © Matsuo-Takasaki, M. et al. 2024. (b) Petri dish of hanging drop culture (the image is self-drawn by the authors). (c) Schematic of the hepatobiliary organoid formation. Reproduced with permission from Ref. [57], © He, J. et al. 2022. (d) Assembly of magnetized cells and spheroid formation using a magnet pin. Reproduced with permission from Ref. [21], © WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 2020.

successful collisions that lead to stable aggregate formation. In larger-scale systems, such as spinner flasks, the impeller design and its rotational speed become the primary generators of shear stress and mixing. These parameters also require careful calibration to minimize cell damage while effectively preventing sedimentation.

3.2.2 *Hanging drop culture methods*

Suspension culture operates on the basic principle of suspending droplets within a liquid culture medium. Cells are then cultured under suitable environmental conditions. The primary advantage of this method is its simple operation. However, it has a significant limitation. The contact area between the cells and the culture medium is limited. This restriction fails to provide adequate nutrient exchange and oxygen supply. Consequently, it limits cell growth and proliferation to some extent. In contrast, the hanging drop method represents an improvement over basic suspension culture. Specifically, culture droplets are added onto the surface of the culture medium or a gel matrix. This adjustment achieves a more uniform cell distribution. It also improves the efficiency of nutrient exchange. The hanging drop method reduces factors that typically limit cell growth. It shows particularly noticeable advantages in promoting cell adhesion and aggregation. Overall, it provides cells with a superior microenvironment.

As illustrated in Fig. 5(b), the hanging drop method relies on specially designed culture plates. These plates have recesses at the bottom to form hanging drops. Each recess contains a cell suspension. The formation of a stable hanging droplet depends on a balance of forces. It is governed by the equilibrium between the liquid's surface tension (γLV), solid–liquid-interfacial tension (γSL), solid-vapor-interfacial tension (γSV) and the gravitational force (Fg = ρgV) acting on the droplet volume. The curvature of the well design is crucial. Often, a hydrophilic rim helps pin the contact line. This ensures the droplet remains suspended without slipping. The droplet volume is a critical parameter; it is typically between 30–50 μL. If the volume is too large, it increases Fg and risks detachment. If it is too small, it heightens the surface-area-to-volume ratio, which accelerates evaporation. Gas-permeable membranes in the plate lid help maintain a humidified atmosphere. This minimizes evaporative water loss to less than 10% over 72 h. Such stability is crucial for osmotic balance and preventing excessive concentration of metabolites. Under the influence of gravity, cells naturally aggregate and form 3D structures [54]. The sedimentation process of cells within the hanging drop follows the principles of Stokes' law ($v = (2r^2g(\rho_{cell} - \rho_{medium}))/9\eta$). Here, the settling velocity (v) is proportional to the square of the cell radius (r). This gravitational settling concentrates cells at the bottom of the droplet, specifically at the drop's apex. This concentration increases the frequency of cell–cell collisions. Subsequently, the minimization of interfacial free energy at the liquid–air interface creates a confined microenvironment. The surface tension of the culture medium, approximately 72 mN/m at 37 °C, acts to minimize the droplet's surface area. This action gently compacts the sedimented cell pellet. The combined action of gravity and surface tension initiates the formation of a compact cell aggregate within just a few hours. During this process, gravity causes cells to gather at the bottom of the droplet. These gathering forms cell populations with 3D structures and promotes intercellular interactions.

Advanced implementations of this technique can introduce rotational motion to the hanging drop plate. This motion generates an inertial focusing effect within the droplet. The rotation creates a secondary flow, known as Dean vortices. This flow counteracts gravitational settling. It focuses cells towards a single, central point instead of allowing them to spread across the bottom. This inertial focusing effect can significantly reduce the time required for initial cell aggregation. It can drop from several hours to under 10 min. The result is the formation of a single, more compact spheroid. This is an improvement over the traditional static hanging drop method, which may yield multiple, less uniform aggregates. Concurrently, the interfacial tension of the droplet regulates the droplet shape. This further influences cell arrangement and self-organization [54]. The geometry of the well plays a key role in determining the final shape of the hanging droplet. The shape can be spherical or more oblate. This geometry defines the curvature of the liquid–air interface. It generates a capillary force or Laplace pressure ($\Delta P = \gamma(1/R_1 + 1/R_2)$) within the forming aggregate. This pressure, though subtle, applies a constant compressive stress on the developing spheroid. This stress promotes tighter cell–cell contacts. It also enhances the expression of adhesion proteins like E-cadherin, which is crucial for spheroid integrity. The synergistic effect of these forces promotes cell self-assembly and the formation of tissue structures. This is achieved by adjusting droplet morphology and cell density. During the maturation phase, the confined space of the droplet and the resulting cell aggregate create an unique microenvironment. Nutrient and oxygen gradients naturally form, with higher concentrations at the aggregate periphery. This gradient, coupled with the mechanical confinement, mimics physiological conditions found *in vivo*. It can direct spontaneous polarization and differentiation of cells. An example is the formation of a lumen in epithelial organoids. The hanging drop method has been widely applied in the cultivation of pancreatic organoids. Recent innovations have further integrated hanging drop principles with microfluidic devices. In these advanced systems, a 3D-printed mold with optimized well geometry ensures droplet stability for several days. This stability occurs without the need for frequent media replenishment. These platforms allow for continuous perfusion of nutrients via microchannels connected to the hanging drop inlet. This perfusion introduces a very low, defined fluid shear stress, typically on the order of 10^{-4} Pa. This subtle stress can further enhance organoid maturation and function. It works by improving nutrient supply and waste removal without disrupting the fragile aggregate.

3.2.3 *Rotary cell culture methods*

The hanging drop method immobilizes cells locally on the surface of a culture medium or gel. While this allows good control over cell aggregation, it has a significant drawback. The lack of dynamic physical stimulation can easily lead to insufficient cellular metabolism. To address this limitation, rotary cell culture was introduced. Rotary cell culture employs a rotating vessel to provide constant liquid flow and mechanical stimulation. This motion keeps cells in a state of continuous tumbling and movement. Consequently, it improves contact between cells and the medium. It also promotes better oxygen exchange and metabolic processes. Compared to the hanging drop method, rotary culture better simulates the *in vivo* fluid environment. It is therefore widely used for cultivating suspension cells.

These systems utilize low-shear rotating culture systems, such as rotary cell culture systems (RCCS) or rotating wall vessels (RWV). They provide a three-dimensional suspension culture environment for cells. The core mechanical principle is to create a state of continuous free-fall for the cells. The culture vessel rotates around a horizontal axis. Gravity pulls the cells and medium downward, while the vessel's rotation provides a counter-acting centripetal force. By precisely calibrating the rotation speed, these two forces are balanced. This results in a net gravitational vector that approaches zero, effectively simulating microgravity conditions (often termed simulated microgravity).

These devices maintain a uniform flow of the culture medium. This ensures cells receive a stable nutrient supply and efficient gas exchange. Inside the rotating vessel, the fluid dynamics are characterized by solid-body rotation. This means the entire fluid mass rotates as a single unit with minimal internal shear or turbulence. Cells primarily experience a very low, uniform fluid shear stress in this laminar flow environment. This stress is typically maintained below 0.1 dyne/cm^2 to prevent mechanical damage to cell membranes. This low-shear environment is crucial for promoting the formation of large, complex 3D cell aggregates. The rotational motion simulates a microgravity environment, which promotes cell growth and distribution in 3D space. Compared to other methods, the low shear force effectively reduces mechanical damage to cells. This increases overall cell viability. Forces like buoyancy and rotational motion work together to reduce gravity's effect. This helps maintain cells in a suspended state [56]. In more advanced systems, such as Random Positioning Machines or 3D clinostats, the rotational forces are more complex. These devices rotate on multiple axes or randomize the direction of rotation. This continuous reorientation prevents cells from experiencing a consistent gravitational pull in any single direction over time, leading to a more effective averaging of the gravity vector to near zero and a more precise simulation of microgravity. The synergistic effect of these forces contributes to uniform cell distribution and tissue formation in 3D space. Rotary cell culture has been successfully applied to the cultivation of brain organoids. By optimizing rotation speed and medium composition, Mirella Dottori et al. have successfully promoted the differentiation and maturation of neural cells, thus providing an effective culture platform for *in vitro* models of neural organoids [56]. Research has also demonstrated the construction of hepatic organoids using rotation. Liver cells were first differentiated into hollow hepatocyte-like organoids (HHOs) using matrix gel embedding. These HHOs were then transferred to a rotating bioreactor with a flowing suspension environment. This step optimized nutrient absorption and facilitated progenitor cell self-assembly into functional organoids. A critical step in this process is initial cell adhesion to microcarriers. In the rotating vessel, cells and microcarriers are kept in suspension. The gentle tumbling motion increases the frequency of cell-bead collisions. Once a cell contacts a bead, adhesion molecules facilitate firm attachment. The rotation speed must be optimized to promote collisions without generating excessive shear that would dislodge newly attached cells. As the technique advances for constructing biliary tract-like organs, careful selection of conditions is imperative. This includes adjusting rotation speed, optimizing culture medium, and incorporating specific growth factors (Fig. 5(c)) [57].

The mechanical regulation of rotary culture has been validated across diverse organoid models. In hepatic organoid construction, the equilibrium between buoyancy-driven suspension and

rotational shear stress enabled sustained hepatocyte polarization. One study found that optimizing rotation speed increased albumin secretion by 3.2-fold compared to static 3D culture. This was achieved through fluid shear-mediated YAP nuclear translocation and bile canaliculi formation [58]. For cardiac organoids, centrifugal force gradients promoted cardiomyocyte alignment. This achieved 89% sarcomere orientation consistency through integrin-mediated mechano-transduction. Furthermore, the rotation establishes critical mass transfer gradients. As cells consume nutrients, concentration gradients form from the surface to the core. The external fluid flow continuously replenishes nutrients at the surface and carries away waste. This dynamic environment mimics the *in vivo* situation more closely than static cultures. It is essential for the survival of cells deep within larger organoids.

3.2.4 *Magnetic levitation culture methods*

While rotary cell culture provides superior conditions, it relies on mechanical rotation. This means the cell growth environment can be affected by equipment design and operational stability. A particular concern is the potential for local non-uniformities in the direction and intensity of the fluid flow. To address this limitation, magnetic levitation culture has emerged and gained wider application. This method uses an external magnetic field to act on magnetic microparticles, such as magnetic beads. This interaction achieves contact-free cell suspension culture. It overcomes the limitations of mechanical movement found in rotary systems. Furthermore, it allows for a more uniform cell distribution and enables higher-precision control of the culture environment.

In this technique, cells are suspended in a magnetic field using magnetic particles. This process provides a three-dimensional culture environment. Commonly used equipment includes magnetic nanoparticles and magnetic field generators. The magnetic particles label the cells, allowing them to be maintained in suspension within the magnetic field. The magnetic field device then applies a stable field to suspend, adjust, and manipulate the cells. This allows the cells to grow freely in the liquid medium, as illustrated in Figure 5(d) [21]. Magnetic levitation facilitates 3D cell assembly by establishing a precise force equilibrium where a magnetic field gradient generates an upward force that counteracts gravitational and buoyant forces to suspend magnetized cells at the air–liquid interface. This "invisible scaffold" prevents cell sedimentation and ensures uniform distribution, while the external field induces dipole–dipole interactions between cells to accelerate intercellular contact and spheroid formation kinetics. For example, iron oxide-labeled bone marrow stromal cells can aggregate within seconds, allowing for the rapid construction of complex, compartmentalized tissue architectures. By providing a low-shear, 3D environment where cells move freely, this synergistic interplay of magnetic and physical forces effectively promotes the structural maturation and functional development of high-fidelity organoids.

An advanced application of this technology is precise spatial patterning. By using patterned magnets or magnetic arrays, specific magnetic field gradients can be created. These gradients guide the assembly of magnetized cells into predefined geometries, such as rings or more complex architectures. This process, sometimes referred to as magnetic bioprinting, demonstrates how magnetic forces can be used for directed assembly beyond simple levitation [59–61].

This gradual evolution reflects a clear progression of cultivation techniques, moving from simple approaches to more sophisticated ones. Table 2 summarizes the characterization of mechanical force in organoid culture. Suspension culture has a localized and static nature. The hanging drop method was optimized upon this foundation by improving cell distribution. However, it still relied on a static culture medium. This limitation meant it was unable to fully address the need for dynamic stimulation. Rotary cell culture significantly enhanced the culture process. It improved the uniformity of the culture environment and increased cellular metabolic efficiency. This was achieved by introducing beneficial liquid flow and mechanical stimulation. Finally, magnetic levitation culture represents a further advancement. Building upon the principles of rotary culture, it utilizes an external magnetic field to achieve contactless cell suspension. This method provides more ideal three-dimensional growth conditions for cells. When comparing the core principles, solid substrates-based organoid culture methods focus on providing physical support or precisely controlling the liquid environment. The primary goal is to promote cell growth and differentiation. Their mechanical effects depend mainly on the interactions between cells and the support materials. In contrast, no substrates-based organoid culture methods focus on controlling cell growth through contactless dynamic mechanics. Their mechanical effects are more clearly manifested in the interaction between fluid dynamics and externally applied forces.

4 Mechanical force in organ-on-a-chip

Organ-on-a-chips have emerged as an advanced *in vitro* platforms that replicate human organ units within microfluidic environments, offering revolutionary potential for drug discovery and disease modeling. However, physiological relevance requires simulating the dynamic mechanical microenvironment [62]. While conventional OoC focus on perfusion and barrier integrity [63], next-generation organoid-on-chip systems recreate the mechanical cues—including shear stress, hydrostatic pressure, matrix stiffness, and cyclic strain—that govern organoid morphogenesis and functional maturation [64].

These mechanical foci are highly organ-specific (Fig. 6): Brain-on-a-chip systems prioritize interstitial flow and blood-brain barrier shear, while lung-on-a-chip models focus on cyclic alveolar strain. Heart-on-a-chip platforms emphasize electromechanical coupling, whereas tumor-on-a-chip designs replicate elevated interstitial pressure and matrix stiffness. Furthermore, kidney-on-a-chip models utilize fluid shear for renal polarization, liver-on-a-chip systems simulate perfusion-driven metabolic zonation, and gut-on-a-chip architectures integrate peristaltic strain to drive villi morphogenesis. Table 3 summarizes the characterization of these mechanical forces in distinct organ-on-chip models.

Among these cues, fluid flow represents a fundamental mechanical signal. Shear stress (τ) can be expressed as: $\tau = \mu \cdot dv/dy$, where μ is the dynamic viscosity and dv/dy is the velocity gradient near the surface. *In vivo,* shear stress typically ranges from 0.1–2 Pa in capillaries and can exceed 10 Pa in large arteries, depending on geometry and flow conditions [65]. In organoid-on-chip systems, however, perfusion and interstitial flow are intentionally reduced to 10^{-3}–10^{-2} Pa—sufficient to sustain nutrient transport without damaging delicate epithelia [66]. This lower level is sufficient to sustain nutrient transport without damaging delicate epithelial structures. Continuous, low-magnitude shear stress has been shown to promote key processes like lumen formation and epithelial

Table 2 The characterization of mechanical force in organoid culture

Substrate mode	Culture method	Primary mechanical forces	Force generation mechanism	Key characteristics of force	Notable biological effects	References
Solid substrates-based organoid culture methods	Scaffold	•Tension/compression •Cell-matrix traction forces •Interstitial flow	•Scaffold material properties •External mechanical loading •Cell contractility	•Static or dynamic •Tunable magnitude •Force transmission via ECM	•Stem cell differentiation •Tissue maturation •ECM remodeling	[10]
	Microcarrier	•Fluid shear stress •Collision forces •Centrifugal force	•Agitation (stirring/rotation) •Fluid dynamics in bioreactor	•Spatially variable •Depends on agitation rate/reactor geometry	•Scalable cell expansion •Potential shear-induced differentiation •Phenotype maintenance	[13]
	Air–liquid interface	•Surface tension • Gravity •Cell-generated forces	•Air–liquid interface physics •Cell activity and ECM deposition	•Relatively static •Interface stability	•Stratified epithelium formation •Barrier function •Mucin production	[52]
	Microfluidics	• Hydrodynamic shear stress •Pressure gradients •Controlled mechanical strain	•Precision fluid pumps •Chip geometry design •Integrated actuators	•Highly controllable •Low Reynolds number flow •Precise spatial control	•Vascular network formation •Organ-on-chip functionality •Physiological drug testing	[14]
	Bioprinting	• Shear stress •Compressive forces •Cell traction forces	•Printing nozzle mechanics •Bioink rheology •Post-printing cell activity	•Transient high stress •Cell-generated forces	•Spatial organization •Long-term construct function	[15]
No solid substrates-based organoid culture methods	Suspension culture	•Gravity •Cell-cell adhesion forces •Buoyancy forces	•Liquid medium •Cell surface adhesion molecules	•Static physical forces •Homotypic cell aggregation dominant	•Spheroid self-assembly •Embryoid body formation •Initiation of differentiation	[55]
	Hanging drop	•Surface tension •Gravity •Cell-cell adhesion forces	•Medium–air interface •Cell surface adhesion molecules	•Static physical forces •Force balance enables spheroid formation	•Embryoid body formation •Initiation of self-assembly •Differentiation cues	[54]
	Rotary culture	•Hydrodynamic shear •Gravity •Cell adhesion forces	•Vessel rotation •RCCS design shear	•Dynamic but low shear •Speed/geometry-dependent	•Complex aggregate formation •Enhanced viability for sensitive cells •Improved differentiation	[56]
	Magnetic levitation	•Magnetic force •Gravity •Fluid drag	•External/internal magnetic fields •Magnetic nanoparticle	•Contact-free force •Static field •Low shear	•Rapid spheroid formation •Enhanced ECM deposition •Microgravity simulation	[21]

polarization. This helps reproduce essential aspects of tissue morphogenesis. Furthermore, the direction and pulsatility of flow can be programmed to model different flow regimes [67].

Matrix stiffness defines another crucial dimension of the mechanical microenvironment. The elastic modulus of native tissues spans 100 Pa in brain tissue to 10,000–50,000 Pa in muscle and cartilage [68]. To replicate this range, organoid-on-chip devices employ tunable hydrogels (e.g., polyethylene glycol (PEG), alginate, gelatin methacryloyl (GelMA)) and elastomeric membranes (e.g., PDMS) [69]. Cyclic strain—typically 5%–15% at 0.5–1 Hz—is applied using pneumatic or magnetic actuation to mimic physiological motions such as cardiac beating or intestinal peristalsis [70]. These dynamic deformations strengthen the cytoskeleton and enhance cell-matrix adhesion. For example, periodic stretching in cardiac organoid chips promotes sarcomere maturation. In gut chips, rhythmic strain can induce villus-like folding [71]. The interplay between stiffness and strain is critical. Soft, viscoelastic matrices dissipate tension over time, minimizing mechanical fatigue. In contrast, stiff elastic substrates accumulate stress and activate mechano-transductive transcription factors [69]. By carefully tuning these parameters, organoid-on-chip systems can emulate developmental processes driven by mechanical feedback.

Beyond shear and strain, hydrostatic and compressive pressures also play indispensable roles in tissue morphogenesis [72]. Pressure gradients can be applied via microvalves or pneumatic chambers. These gradients simulate conditions like vascular perfusion or tissue compression [16]. Typical pressures used range between 0.5–5000 Pa, approximating physiological interstitial conditions. Pressure modulation influences processes like morphogen transport and lumen expansion [73]. Sustained pressure supports cystic organoid growth, while pulsatile compression can trigger differentiation. Integrating pressure control with real-time imaging allows researchers to correlate mechanical load with morphological changes [74]. In parallel, integrating on-chip pressure readouts or flow sensors enables continuous quantification of the applied load, which helps distinguish true biological responses from device-to-device variations.

Native tissues exhibit viscoelastic rather than purely elastic behavior, characterized by stress relaxation and energy dissipation[75]. To capture this, modern systems incorporate adaptive hydrogels. The relaxation dynamics of these materials can be tuned independently of stiffness. Biological matrices typically exhibit relaxation times of 10–100 s. This timescale aligns with cytoskeletal remodeling. Viscoelastic environments allow organoids to deform without accumulating residual stress. This improves long-term culture stability. These platforms are also versatile for studying dynamic phenomena like mechanical memory, which is critical in disease modeling [67].

Over the past decade or so, the development of organoid and OoC technologies has gone through a series of milestones, as systematically chronicled in this dual-timeline infographic (Fig. 7). Mechanical forces play a pivotal role in OoC evolution: The 2010

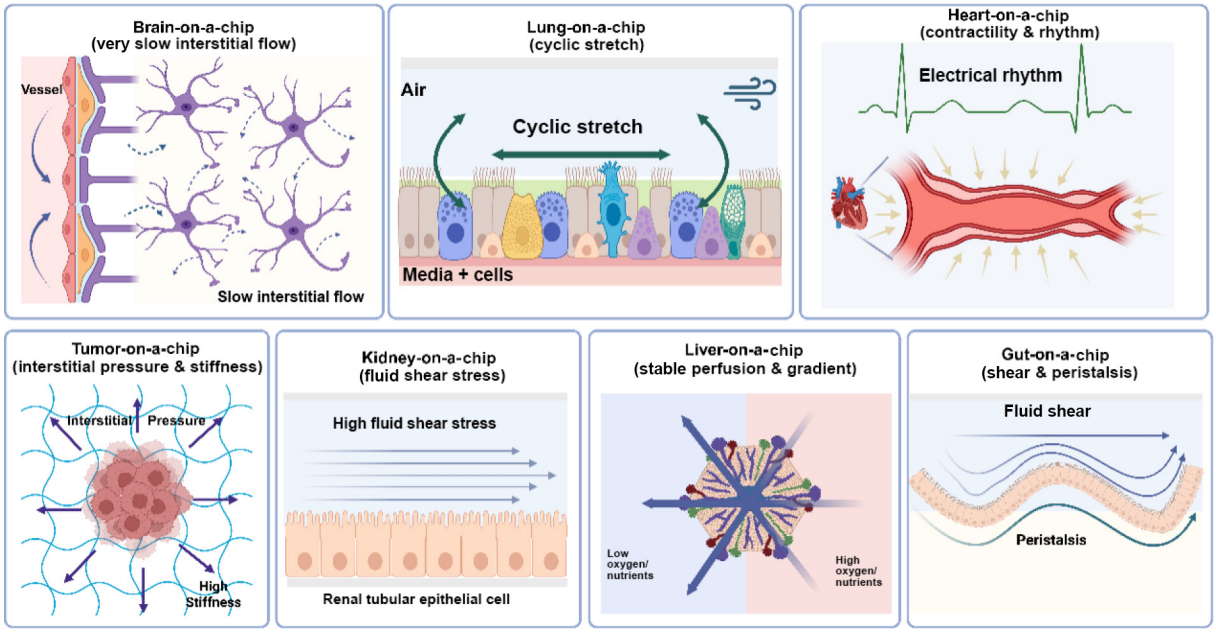

Figure 6 Organ-on-a-chip mechanical force simulation focus. The images are self-drawn by the author, with some illustration prepared using BioRender.com. Copyright BioRender, 2026.

lung-on-a-chip pioneered biomechanical alveolar stretching, while the 2012 gut-on-a-chip breakthrough integrated *Fluid Shear* stress and *Peristalsis* to mimic intestinal motility. By 2015, kidney chips leveraged fluid dynamics to replicate renal tubular filtration functions, and 2018's BBB-on-a-chip engineered endothelial barrier responses through controlled shear stress. In contrast, organoid advancements emphasized microenvironment control—the 2019 vascularized organoid achieved perfusion through engineered vasculature, enabling nutrient transport in thick tissues. This timeline demonstrates how mechanical forces are indispensable for organoids and OoC.

4.1 Brain-on-a-chip

The brain, as the control center of the vertebrate nervous system, processes information and regulates bodily functions through complex neural networks. Its physiological activities rely on multiscale mechanical interactions. These range from nanoscale synaptic tension regulating neurotransmitter release, to macroscopic cerebrospinal fluid pulsations modulating neural homeostasis. Such hierarchical mechanics not only sustain brain function but also inspire the design of brain-on-a-chip systems. In these systems, force transmission, signal processing, and metabolic coupling are interlinked.

Since the late 2000s, researchers have progressively reconstructed the brain's physicochemical microenvironment and mechanical stimulation systems within chips. Early BoC systems primarily modeled the blood-brain barrier. They used 2D endothelial monolayers exposed to laminar shear stress to reproduce vascular mechanical responses. However, these planar configurations oversimplified the three-dimensional coupling between vascular and neural compartments. This limitation restricted their ability to reproduce the dynamics of the neurovascular unit. From the mid-2010s, the introduction of multi-cell co-culture techniques led to composite layered structures. These structures incorporated endothelial cells, astrocytes, and pericytes, significantly improving the physiological fidelity of BBB models. For example, a system developed by the University of Leeds team applied laminar shear stress through microfluidic channels. It also coupled hydrostatic pressure to simulate the brain tissue microenvironment. This approach successfully validated how shear stress and pressure work together to regulate the expression of tight junction proteins like ZO-1 and Claudin-5 [77]. As shown in Fig. 8(a), the goal of the device construction is to apply a controlled flow rate to culture brain endothelial cells surrounded by astrocyte endpods. The device and endothelial channels are within the hydrogel. The chip has two isolated compartments, represented by dye infusion. Flow and hydrostatic pressure drive cyclic strain, which regulates the pressure at the inlet and outlet of the equipment [78]. Despite these advances, such 2D models still had limitations. They were unable to replicate the dynamic interactions of the full neurovascular unit; for instance, the regulatory role of pericytes on endothelial barrier function remained difficult to fully manifest in static culture [79].

Towards the late 2010s, the integration of 3D cell culture with microelectrode arrays (MEAs) spurred innovations in analyzing neural network function (Fig. 8(b)). For example, a team from Zhejiang University coupled brain organoids with MEAs, successfully simulating inter-neuronal electrical signal transmission and the mechanosensitive properties of glial cells through a combination of electric field stimulation and local shear stress. Experiments demonstrated that electric field stimulation could induce transient increases in neuronal calcium ion concentration, while shear stress enhanced glial cell β-catenin signaling pathway activity via Piezo1 channels [80]. However, limitations persisted. The 2D electrode layouts restricted large-scale dynamic monitoring of neural circuits. To address these spatial recording and long-term culture limitations, a next-generation platform incorporated a dedicated three-dimensional modular system for modeling neurodegenerative diseases, as illustrated in Fig. 8(c). This core module, represented as a transparent hydrogel construct, integrates six critical aspects for faithful disease modeling: immune competence, a tunable microenvironment, brain-like extracellular matrix, structural complexity, culture robustness, and dynamic fluid

Table 3 The characterization of mechanical forces in distinct organ-on-chip models

Organ chip model	Simulated system/focus	Dominant force (s)	Physiological/pathological relevance	Force characteristics	Method of force application	Representative Reference(s)
Brain chip model	Blood-brain barrier (BBB) model	Fluid shear stress (FSS)	FSS induces/maintains BMEC barrier properties; regulates transporter expression/function.	FSS: 0.04–0.5 Pa, 0.4–5 dyn/cm^2, sometimes higher up to 1–1.5 Pa pattern: Laminar, can be steady or pulsatile.	Perfusion of medium using peristaltic, syringe pumps, or pressure controllers through the microfluidic channel on the apical side (vascular lumen) of the endothelial monolayer.	[115, 116]
	Neurovascular unit (NVU) Model	FSS Interstitial flow (IFF)	FSS on endothelium affects NVU cell interactions. IFF aids waste clearance.	FSS: same as BBB model. IFF: flow rates: ~ 0.1–0.5 μm/s.	FSS: vascular channel perfusion. IFF: pressure difference between compartments or slow perfusion through 3D matrix.	[115, 117]
	Traumatic brain injury (TBI) model	Rapid mechanical strain Stress Shear stress	TBI rapid deformation causes primary injury and triggers secondary injury.	Strain/stress: 10%–50% strain, rate: ms scale. Pattern: tensile/compressive/shear.	Application of impact/stretch/compression/shear to cell culture substrate or 3D construct using rapid actuators (e.g., pneumatic, electromagnetic).	[118, 119]
Lung chip model	Alveolar model	Cyclic mechanical stretch Strain Vascular FSS	Mimics breathing and maintains alveolar structure. FSS: capillary flow on endothelium.	Stretch: 5%–15%, frequency: 0.2–0.5 Hz. Pattern: biaxial or uniaxial FSS: 0.05–0.2 Pa. Pattern: laminar/pulsatile.	Stretch: vacuum/pressure-driven deformation of flexible PDMS membrane; direct mechanical stretching of substrate. FSS: perfusion through microchannel (vascular side).	[16]
	Airway bronchiole model	FSS-airflow Mucus flow Air-liquid interface	FSS: aids mucociliary clearance/epithelial differentiation/ion transport/barrier. ALI: physiological gas exposure/cell differentiation.	FSS: ~ 0.001–0.05 Pa, Pattern: laminar/low oscillatory flow ALI: apical exposure to air, basal contact with medium	FSS: generated by airflow in upper channel/liquid perfusion in lower channel; use of rocking platform for oscillatory flow. ALI: culture on porous membrane.	[120, 121]
	Pulmonary fibrosis model	Aberrant mechanical forces	Fibrosis: ECM deposition/ stiffening/exacerbate fibrosis.	Stretch: use pathologically relevant higher strains or frequencies. Matrix stiffness: high stiffness (> 10–20 kPa) hydrogels mimicking fibrotic matrix.	Stretch: alveolar model. Stiffness: use of tunable hydrogels or PDMS.	[122]
Tumor chip model	Solid tumor core Compression model	Compressive stress Solid stress	Tumor pressure/internal stress impact proliferation/vessel collapse/drug penetration/invasion.	Stress: 1–10 kPa Strain: variable Pattern: static/dynamic compression	External piston/actuator; confining culture wells; endogenous pressure from spheroid growth or hydrogel swelling.	[123, 124]
	Matrix interaction Stiffness model	Matrix stiffness Tension	ECM stiffening impacts migration/invasion/EMT drug resistance/CAF contractility.	Stiffness: Young's modulus: 0.5 kPa–40+ kPa Tension: measured via traction force microscopy or flexible pillars	Tunable hydrogels; flexible micropillar arrays; External stretching.	[125, 126]
	Tumor vasculature Metastasis model	Intravascular FSS	Abnormal FSS impacts endothelial barrier/angiogenesis/CTC survival/adhesion/extravasation.	Magnitude: 0.1–1 Pa Pattern: laminar/low oscillatory/irregular flow	Perfusion of endothelial layers in microfluidic channels.	[127, 128]
	Immuno-oncology model	IFF FSS Matrix stiffness	IFF affects immune trafficking. FSS affects endothelial-immune interactions. Stiffness affects immune activity/infiltration.	Combined depending on model focus	Perfusion; matrix stiffness tuning; co-culture systems.	[129]
Kidney chip model	Glomerular model	Transmural pressure gradient FSS	Pressure gradient drives ultrafiltration; forms primary urine; GFR. FSS affects podocyte/EC structure/filtration barrier integrity.	Pressure gradient: 10–50 mmHg, 1.3–6.7 kPa. Pattern: static/pulsatile. FSS: 0.1–1 Pa. Pattern: laminar/pulsatile.	Pressure gradient: established across a porous membrane by controlling inlet/outlet pressures. FSS: pump-driven perfusion within the vascular channel. Filtration membrane: often porous PDMS or polycarbonate membrane coated with functionalized or native ECM to mimic GBM.	[130, 131]
	Proximal tubule model	FSS	FSS maintains PT cell differentiation/polarity, reabsorption, cilia, drug transport/metabolism.	FSS: ~ 0.01–0.2 Pa, 0.1–2 dyn/cm^2 Pattern: laminar/steady flow.	Perfusion of medium using peristaltic, syringe pumps or gravity flow.	[132, 133]

(Continued)

Organ chip model	Simulated system/focus	Dominant force (s)	Physiological/pathological relevance	Force characteristics	Method of force application	Representative Reference(s)
Heart chip model	Distal tubule Collecting duct model	FSS Osmotic gradient	FSS: distal nephron flow affects cell morphology, ion transport, hormone response. Osmotic gradient: drives ADH-regulated water reabsorption.	FSS: generally lower than PT, ~ 0.001–0.02 Pa. Pattern: laminar/steady flow. Osmotic gradient: established by altering basolateral medium osmolality.	FSS: as in PT models, but lower flow rates. Osmotic gradient: use of osmolytes in contra luminal medium.	[134]
	Myocardial tissue model	Cyclic mechanical stretch Strain Electrical stimulation (E-Stim)	Stretch: mimics diastole/systole; drives CM alignment, maturation, contractility, gene expression. E-Stim: mimics pacing; coordinates contraction; affects electrophysiology.	Stretch: 5%–20%. Frequency: 1–2 Hz. Pattern: uniaxial/biaxial. E-Stim: 1–5 V/cm, 1–10 ms pulse width. Frequency: 1–2 Hz. Pattern: field stimulation for synchronous pacing	Stretch: deformation of flexible substrate via vacuum/pressure/actuator; tissue anchored between/on movable flexible posts/cantilevers. E-Stim: integrated electrodes; external electrodes inserted into medium.	[95, 96]
	Cardiac fibrosis model	Cyclic mechanical stretch Matrix stiffness modulation	Stretch activates CFs; myofibroblast transformation; ECM deposition; tissue stiffening. Stiffness influences CFs.	Stretch: 5%–15%. Frequency: 1–1.5 Hz. Matrix stiffness: tunable range, 5–20+ kPa	Stretch: as above. Stiffness: use of hydrogels with varying crosslinking.	[135, 136]
	Cardiovascular interaction model	FSS Cyclic stretch	FSS (intracardiac flow) on endocardium; endothelial phenotype; barrier; myocyte interaction.	FSS: 0.1–1 Pa. Pattern: pulsatile/oscillatory. Stretch: magnitude/frequency may be adjusted for model.	FSS: perfusion of fluid through channel over endothelial layer. Stretch: endothelial cells cultured on stretchable substrate.	[100]
Liver chip model	Liver sinusoid model	FSS	FSS maintains LSEC phenotype, hepatocyte polarity/metabolism, regeneration, inflammation, cell communication.	FSS: 0.01–0.1 Pa, 0.1–1 dyn/cm^2. Pattern: laminar/steady flow.	Perfusion using peristaltic/syringe pumps/gravity flow within microfluidic channels lined with hepatocytes and/or multiple cell types.	[137, 138]
	Bile canaliculi bile flow model	Bile flow Shear stress	Osmotic gradient drives bile flow; maintains canalicular structure, TJs, bile transporter function.	Flow/FSS: very low, difficult to directly measure/control precisely, often generated by cell's own secretory activity (<< 0. 01 Pa).	Primarily relies on hepatocyte function to form sealed canalicular networks and osmotic gradients. Extremely low flow can also be applied via microchannels.	[139, 140]
	Liver fibrosis NASH model	Matrix stiffness FSS Inflammatory stimuli	Fibrosis/NASH: ECM deposition, stiffening; increased stiffness activates HSCs; affects hepatocytes, inflammation.	Matrix stiffness: Young's modulus: 1–5 kPa to > 10–40 kPa. FSS/inflammatory stim: may combine low FSS and/or addition of fatty acids, LPS, cytokines.	Stiffness: use of tunable hydrogels (e.g., collagen, fibrin, hyaluronic acid, GelMA, PEG) as culture substrate. Other stimuli: perfusion or static addition of chemical/biological stimuli.	[141]
Intestine chip model	Small intestine model	FSS Cyclic mechanical strain Stretch	FSS: Luminal flow influences villi, polarity, differentiation, barrier, absorption, mucus, microbe interaction. Strain: peristalsis influences villi, proliferation, differentiation, barrier, mucus, immune trafficking.	FSS: 0.001–0.02 Pa, 0.01–0.2 dyn/cm^2. Pattern: laminar/simulating slow flow. Strain: 5%–15%, frequency: 0.1–0.3 Hz, 6–18 cycles/min. Pattern: biaxial/uniaxial.	FSS: peristaltic or syringe pump drives medium flow through apical channel. Strain: vacuum/pressure drives cyclic deformation of flexible PDMS membrane.	[142, 143]
	Colon model	FSS Cyclic strain ALI	FSS: low colonic FSS affects epithelial function, barrier, mucus. Strain: colonic motility. ALI: proximal colon low oxygen/gas exposure.	FSS: 0.001–0.01 Pa. Strain: Mag/Freq can be adjusted based on colonic motility characteristics. ALI: apical exposure to specific gas environment.	FSS/strain: as in small intestine models. ALI: removal of apical medium, control of overlying gas.	[144, 145]
	Intestinal vascular barrier model	FSS Luminal Vascular side	FSS: luminal on epithelium; vascular on endothelium. Nutrient absorption; drug transport; leukocyte trafficking.	Luminal FSS: 0.001–0.02 Pa Vascular FSS: 0.05–0.5 Pa	Perfusion of upper and lower channels separately in a two-layer microfluidic chip separated by a porous membrane, with intestinal epithelial cells and endothelial cells cultured on opposite sides.	[146, 147]

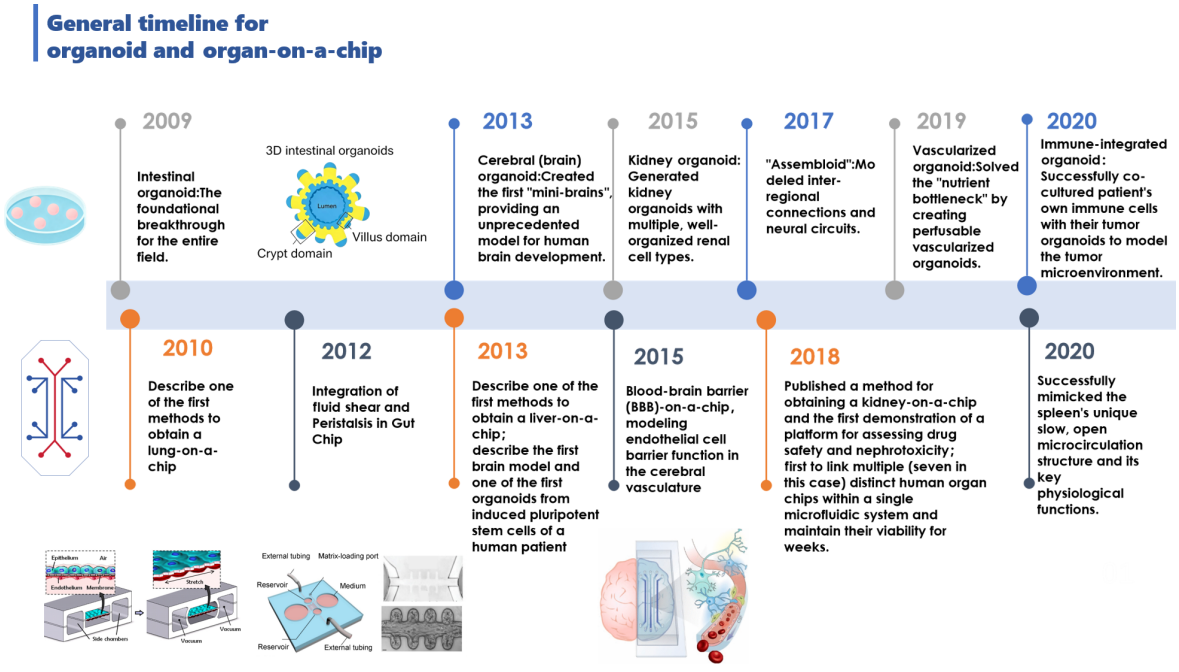

Figure 7 General timeline for organoid and OoC. Reproduced with permission from Ref. [16], © American Association for the Advancement of Science 2010. Reproduced with permission from Ref. [76], © Fang, G. C. et al. 2023. Reproduced with permission from Ref. [79], © American Chemical Society 2020. Some images are self-drawn by the authors.

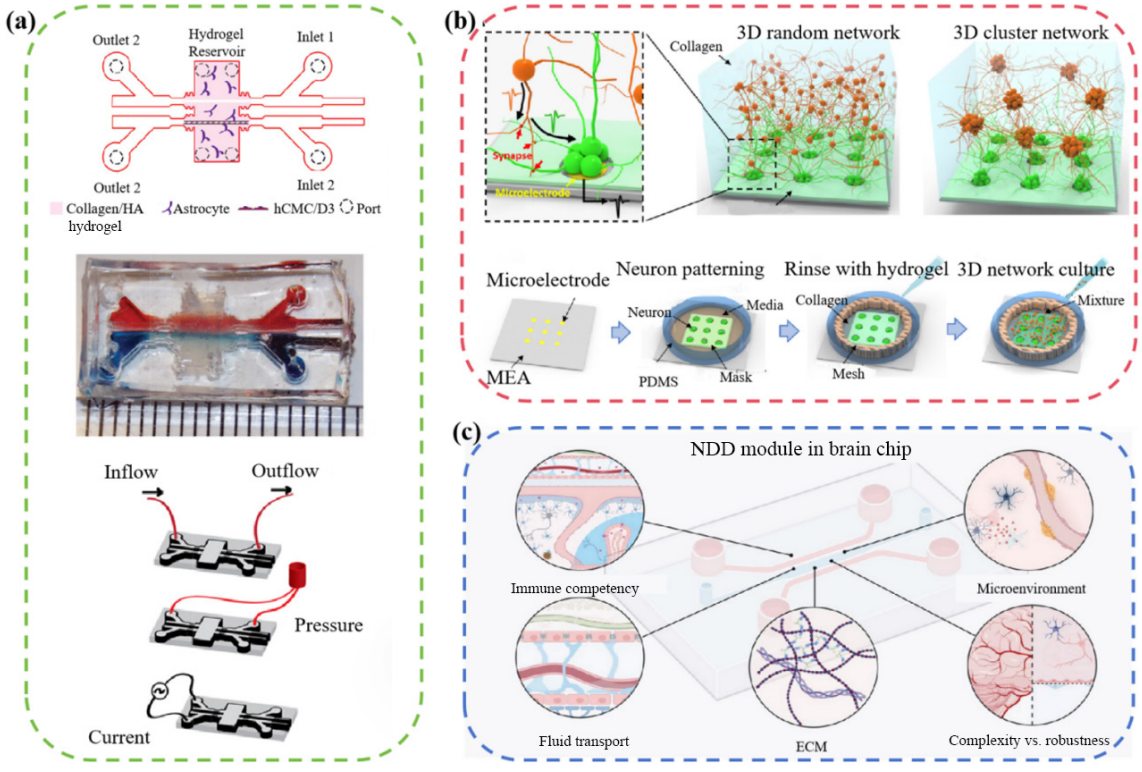

Figure 8 (a) A BBB model. Reproduced with permission from Ref. [78], © Mir, M. et al. 2022. (b) Construction of 3D neuronal networks with neural cluster interfaces on microelectrode arrays. Reproduced with permission from Ref. [83], © Elsevier B.V. 2024. (c) Graphical NDD models. Reproduced with permission from Ref. [84], © Pramotton, F. M. et al. 2024.

transport. This integrated approach enables the sustained culture and functional analysis of complex 3D neuronal networks under conditions that closely mimic the pathological microenvironment, directly addressing the metabolic and monitoring shortcomings of prior systems.

Entering the 2020s, highly biomimetic systems achieved precise control over the brain's multi-dimensional mechanical environment. They integrated multi-layer microfluidics, flexible substrate deformation, and multi-modal sensing technologies. This resulted in organoids that exceeded traditional size limits with

significantly reduced hypoxic apoptosis rates [81]. However, existing platforms still face technical bottlenecks such as insufficient long-term culture stability (high rates of organoid core necrosis) and significant inter-individual variability [81, 82]. Future development focuses on two main directions. First, researchers will develop alginate-gelatin composite bioinks for personalized customization of vascularized microenvironments. Second, they will dynamically optimize shear stress frequency using AI algorithms based on deep reinforcement learning. These two strategies work together to address core bottlenecks. Vascularization engineering overcomes diffusion limitations of nutrients and oxygen by bioprinting customizable vasculature networks. This resolves the necrotic core issue common in traditional larger organoids, establishing a survival foundation for sustaining complex neural networks long-term. AI-driven dynamic mechanical optimization uses deep reinforcement learning to precisely regulate shear stress patterns in real-time. This accurately simulates the hemodynamic microenvironments that govern neurogenesis and synaptic plasticity in the brain, for example, significantly increasing oligodendrocyte maturation rates.

4.2 Lung-on-a-chip

The lung is the most critical organ in the respiratory system of terrestrial and many aquatic or semi-aquatic animals. The fundamental functional unit of the lung is the alveolus. Alveoli have thin epithelial walls and are surrounded by dense capillary networks. This structure enables rapid and efficient gas exchange. During respiration, alveoli undergo periodic expansion and contraction. This movement imposes rhythmic mechanical loads on the surrounding tissues. Therefore, lung-on-a-chip systems are designed to reproduce two key aspects. First, they replicate the gas exchange interface. Second, they simulate the dynamic mechanical stresses occurring at the ALI. These stresses typically involve cyclic strain of about 5% to 15% at frequencies of 0.2 to 0.3 Hz. They are also accompanied by vascular shear stress on the order of 10^{-3} to 10^{-1} Pa [85]. These physical parameters are crucial for maintaining alveolar homeostasis. They are also vital for accurate biomechanical modeling.

In 2010, Huh et al. [16] developed a micro-device that marked a milestone upgrade in the mechanical systems of lung-on-a-chip (Fig. 9(a)). The research team designed a dual-layer microfluidic device. It used a porous, elastic PDMS membrane to separate an air chamber from a fluid chamber. Periodic negative pressure was applied through side vacuum channels. This generated tensile deformation of approximately 10% to 15% on the membrane. This action closely mimicked alveolar expansion and contraction during breathing. The rhythmic strain activated ion channels in alveolar epithelial cells. It also promoted ECM remodeling. When epithelial and endothelial cells were cultured on opposite sides of the same

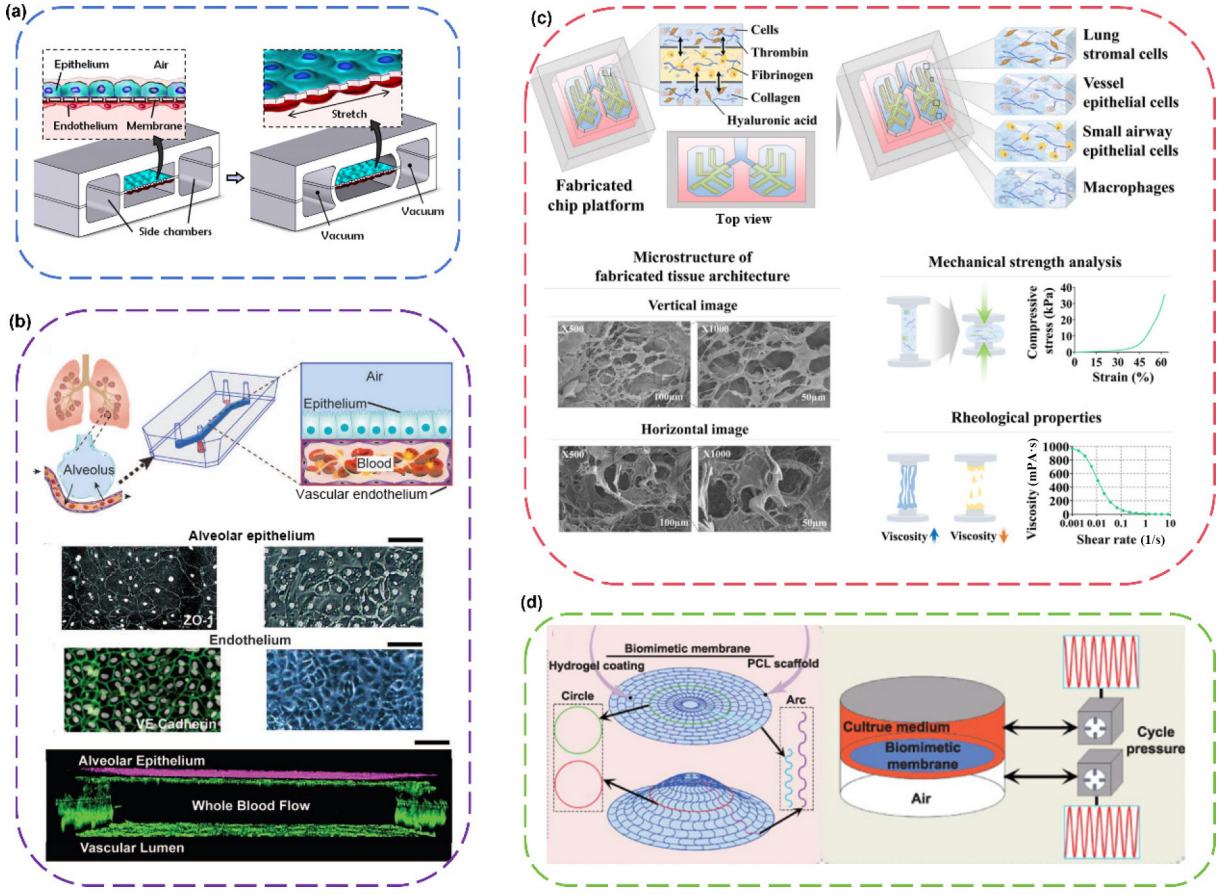

Figure 9 (a) Biologically inspired design of a human breathing lung-on-a-chip microdevice. Reproduced with permission from Ref. [16], © American Association for the Advancement of Science 2010. (b) Microengineered model of human pulmonary thrombosis-on-chip. Reproduced with permission from Ref. [86], © Wiley-VCH GmbH 2017. (c) Basic concept of human lung-on-a-chip that accurately mimics structural and multicellular complexities of lung tissue. Fabrication of natural polymers-based lung tissue constructs and examination of its physical properties. Reproduced with permission from Ref. [90], © Min, E. K. et al. 2025. (d) A microengineered model of the biomimetic controllable strain membrane (BCSM) for cell stretching and the working principle of the biomimetic lung unit model. Reproduced with permission from Ref. [88], © Min, E. K. et al. 2023.

membrane, moderate deformation enhanced the alveolar-capillary barrier function. In contrast, excessive stretching beyond 20% induced rupture and edema-like leakage. This reflected mechanical injury thresholds observed in ventilator-induced lung damage. This work first demonstrated that the magnitude and frequency of respiratory strain directly determine molecular injury responses.

After 2015, the mechanical systems of lung-on-a-chip entered a phase of multi-dimensional integration. Researchers began coupling multiple types of mechanical forces within a single chip. For example, they simulated alveolar respiratory motion. At the same time, pulsatile blood flow in the vascular channel was controlled using microfluidic pumps. This subjected endothelial cells simultaneously to shear stress and basement membrane stretching. In the context of these advancements, a landmark study by Jain et al. introduced a primary human lung alveolus-on-a-chip model specifically designed to study intravascular thrombosis and assess therapeutics (Fig. 9(b)). It illustrates a microengineered human lung alveolus-on-a-chip that reproduces the mechanically dynamic alveolar–capillary interface by combining defined microchannel geometry with controlled fluid shear and a deformable, ECM-coated porous membrane. The device consists of two parallel PDMS microchannels separated by a thin, flexible membrane, with primary human alveolar epithelial cells cultured on the upper, air-facing side and vascular endothelial cells lining all four walls of the lower, blood-facing channel to create a 3D, compliant microvessel exposed to physiologically relevant whole-blood flow-induced shear stresses. This architecture establishes distinct yet mechanically coupled compartments, in which apical epithelial surfaces experience low shear and maintain barrier tension while the endothelial lumen is subjected to sustained laminar shear and transmural mechanical cues transmitted through the shared membrane, thereby mimicking the spatially organized force environment of the native alveolar wall. Confocal and fluorescence micrographs confirm that both the epithelial and endothelial layers form continuous junctional networks (ZO-1 and VE-cadherin, respectively) over days of perfusion, demonstrating that the engineered tissue–tissue interface can preserve structural integrity and intercellular tension under chronic flow and pressure loading, and thereby provide a mechanically stable platform for studying how hemodynamic forces modulate inflammation-induced thrombosis in a human-relevant alveolar microvascular niche [86].

Recent developments have further focused on the refined control and dynamic response of mechanical parameters. For instance, researchers discovered that photopolymerizable hydrogels could mimic the mechanical properties of healthy and fibrotic lung tissues. They prepared hydrogels with varying stiffness and chemical compositions to simulate the lung tissue microenvironment (Fig. 9(c)). Subsequently, bioreactors were used to apply cyclic strain to the hydrogels, mimicking the mechanical stimuli experienced by the lungs during breathing and mechanical ventilation, revealing the driving role of mechanical heterogeneity in pulmonary fibrosis [87]. Professor Yong He's team [88], inspired by the art of paper cutting (Kirigami), designed and fabricated mesh structures on a planar surface capable of controlling 3D strain through the mesh geometry (Fig. 9(d)). They constructed a biomimetic alveolar organ chip with localized mechanical control capabilities, simulating important structures and functions of the basement membrane. The device's polycaprolactone (PCL) mesh, coated with hydrogel, was patterned with concentric circular and curved geometries, allowing differential strain distribution ranging from 2% to 15%—comparable to the physiological deformation range of alveoli.

Currently, the mechanical systems in lung-on-a-chip are evolving towards intelligence and clinical translation. Machine learning algorithms are being employed to optimize combinations of mechanical force parameters [89]. For instance, feedback regulation can automatically match stretching frequencies to different pathological states. This evolution continues to drive innovation in respiratory disease research and therapeutic approaches.

4.3 Heart-on-a-chip

The heart serves as the central organ responsible for sustaining life activities in the human body. However, the heart's profound complexity far exceeds the simulation capabilities of traditional research tools. Within its dynamic microenvironment, the interplay between mechanical stimuli, like cyclic stretch, and electrical signal conduction is crucial for its function. Among existing research methods, animal models face significant limitations. Due to species differences, such as a mouse heart rate being six times that of humans with differing ion channel sensitivities, they struggle to accurately predict human cardiac responses to drugs (Fig. 10(a)) [91, 92]. Meanwhile, two-dimensional cell culture fails to replicate the heart's three-dimensional structure and mechanical environment. This often leads to missed detection of drug toxicity or distorted insights into disease mechanisms. These limitations underscore the necessity of developing heart-on-a-chip systems.

Initial heart-on-a-chip designs focused on simple microfluidic structures. They primarily comprised single-layer channels and basic electrode structures. Their goal was to simulate fundamental cardiomyocyte functions, such as contraction and electrical activity [93]. In early designs, flow-induced shear stress within the microchannels was used to mimic the hemodynamic influence of blood flow on cardiomyocytes. This shear stress generally ranged from 0.002 to 0.02 Pa [94]. However, research rapidly evolved toward more complex platforms to accurately replicate the heart's multicellular architecture. The focus shifted to multi-cell, multi-force platforms that better simulate the coupled electromechanical environment.

Importantly, the native *in vivo* cardiac microenvironment is defined by coupled stimuli rather than isolated single-factor loading: Blood flow imposes shear stress, intraluminal pressure generates cyclic stretch/strain and wall stress, and the myocardium simultaneously operates under excitation–contraction coupling driven by electrical activation. Because shear and stretch/pressure can converge on shared mechanotransduction programs in cardiovascular tissues, the cellular and tissue responses under combined loading may not be predicted by simply adding the effects of each stimulus applied separately. From an engineering perspective, reproducing this coupling in heart-on-a-chip systems typically requires synchronized integration of perfusion control (to impose defined shear and mass transport), mechanical actuation (e.g., flexible membranes/substrates for cyclic strain and load-like constraints), and electrical pacing via embedded/external electrodes to regulate beat rate and promote maturation.

Ken Takahashi et al. utilized OoC technology to culture induced pluripotent stem cell (iPSC)-derived cardiomyocytes (iPSC-CMs), fibroblasts, and ECs, constructing a heart-on-a-chip (Fig. 10(b)) [95]. Figure 10(b) depicts the mechanically relevant features of the heart-on-a-chip system and the shear stress-dependent response of

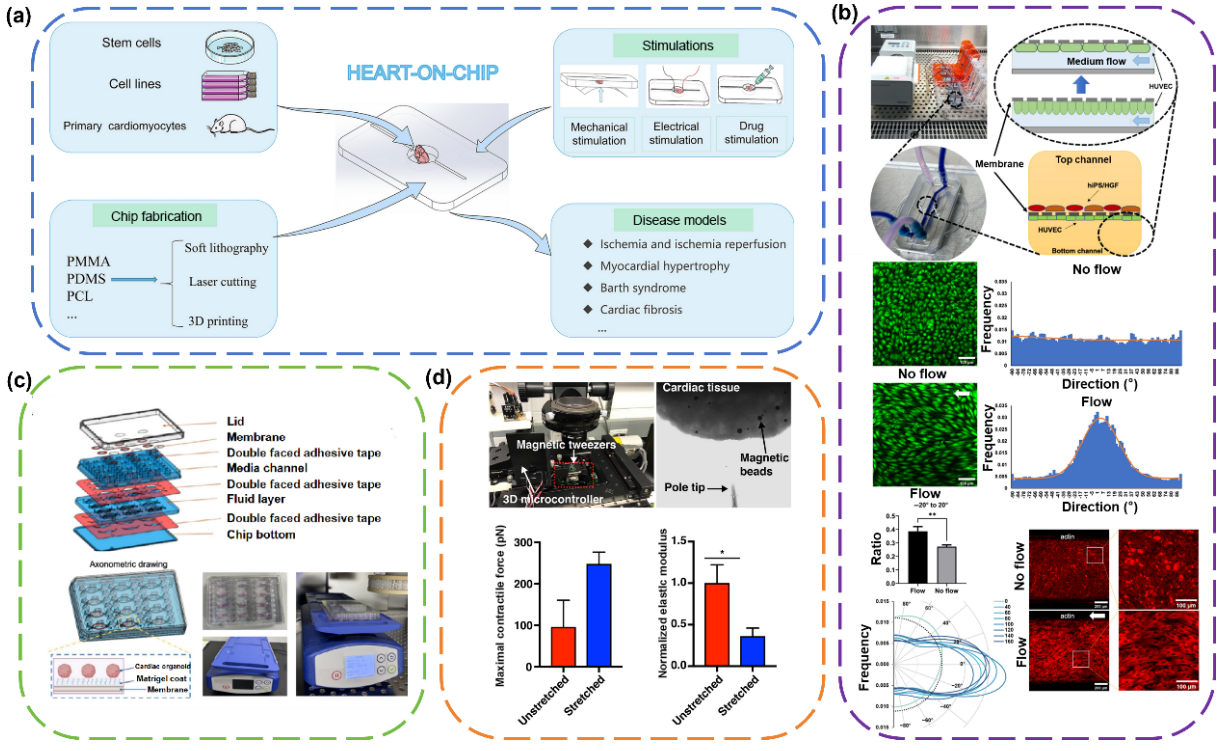

Figure 10 (a) Chip fabrication, cell sourcing, stimulation modules, disease modeling in heart-on-chip systems. Reproduced with permission from Ref. [100], © Liu, B. Q. et al. 2024. (b) Perfusion system for the heart-on-a-chip and the response of vascular endothelial cells to medium flow. Reproduced with permission from Ref. [95], © Liu, Y. et al. 2024. (c) Design of COoC. Reproduced with permission from Ref. [97], © American Chemical Society 2024. (d) Mechanical stimulation improves flexibility and increases maximal contractile force in engineered heart tissue grafts. Reproduced with permission from Ref. [98], © John Wiley & Sons Ltd. 2021.

endothelial cells within the vascular compartment. The dual-channel microfluidic device, comprising a perfused vascular channel and a myocardial channel separated by a porous membrane, is designed to impose a well-controlled level of fluid shear stress on the human umbilical vein endothelial cell (HUVEC) monolayer through continuous medium perfusion, thereby recapitulating key aspects of hemodynamic mechanical stimulation encountered by vascular endothelium *in vivo*. Under these defined flow conditions, endothelial cells undergo characteristic shear-dependent remodeling, as shown by their progressive elongation and alignment in the direction of flow and by the reorganization of F-actin into prominent, flow-aligned stress fibers, with quantitative analyses demonstrating increased orientational order at higher flow rates. Importantly, this dual-channel configuration also illustrates a coupled cardiac-relevant mechanical scenario, where perfusion-derived shear in the vascular compartment can be transmitted across the porous membrane to influence the adjacent myocardial microenvironment via paracrine exchange and interfacial mechanics. Such cross-compartment coupling better reflects the *in vivo* situation in which vascular shear and wall stress/stretch jointly regulate cardiovascular cell phenotypes, rather than acting as isolated single-factor inputs.

Building on this concept, Arun Sharma et al. developed a heart-on-a-chip model based on OoC technology [96]. The chip comprises two fluidically independent channels separated by a porous membrane, enabling paracrine communication and diffusion between cell layers. hiPSC-derived cardiomyocytes and endothelial cells were subjected to rhythmic deformation at approximating human heartbeat frequency with 10% contraction amplitude and continuous medium perfusion at 30 μL/h. Under these conditions, α-actinin-GFP–expressing cardiomyocytes showed well-aligned sarcomeres and stronger contractile force, indicating enhanced maturation. This platform represents a typical multi-mechanical coupling regime in heart-on-a-chip, where rhythmic deformation (strain/loading) is applied concurrently with continuous perfusion (shear and mass transport), enabling responses that may not be reproduced under deformation-only or perfusion-only conditions.

While the aforementioned models significantly advanced the simulation of tissue–tissue interfaces and combined mechanical forces, they are often based on planar cell layers. The next logical step towards achieving higher structural fidelity has been the integration of self-assembling, 3D tissue constructs, which more closely mimic the native architecture of the myocardium. A prime example of this approach is the work by Liang et al. [97], which integrated cardiac organoid (CO) and OoC technologies to develop a cardiac-organoid-on-a-chip (COoC) platform (Fig. 10(c)), which was validated for cardiotoxicity assessment across multiple dimensions. The team utilized a high-throughput, membrane-based universal chip featuring independent upper and lower flow channels, separated by a biocompatible porous membrane. This configuration enabled interaction between cells in the upper and lower layers via the membrane, thus constructing an interface structure mimicking the human tissue-tissue barrier. Sustainable fluid flow within the chip was achieved through gravity-driven perfusion, facilitating sufficient exchange of oxygen and nutrients. Furthermore, a rocking perfusion system was employed to simulate the biophysical characteristics of tissues using shear stress generated by the culture medium, providing hydrodynamic conditions for organoid culture on the chip to emulate the *in vivo* growth environment. For cardiac organoids, perfusion/rocking introduces not only nutrient exchange but also hydrodynamic shear, which

couples with the organoid's 3D architecture and intrinsic contractility to shape maturation trajectories and drug-response readouts. Moving forward, integrating controllable pressure or load-like constraints (preload/afterload surrogates) together with perfusion would further strengthen the multi-force coupling fidelity of cardiac-organoid-on-chip systems. OoC technology enables the creation of micro-physiological environments, allowing precise control over tissue interfaces and fluid shear stress through tissue engineering and microfluidics. Compared to static culture, mechanical loading in OoCs significantly promotes cardiomyocyte and organoid maturation (Fig. 10(d)) [98].

These examples highlight the remarkable progress in recapitulating cardiac complexity. The core functionality of these systems lies in their ability to precisely recapitulate the heart's mechanical microenvironment. However, achieving this goal remains challenging due to multiple technical barriers. A significant challenge is the biomimetic reconstruction of the heart's dynamic mechanical environment. Currently, the mechanical properties of scaffold materials, such as elastic modulus and viscoelasticity, are insufficiently matched to those of native cardiac tissue. This limits the mechanical responsiveness of cardiomyocytes [99]. For instance, existing scaffolds often fail to replicate the natural mechanical characteristics of the extracellular matrix. This adversely affects cell alignment and contractile function. Furthermore, optimization of dynamic mechanical stimuli, such as cyclic stretching and fluid shear stress, requires advancement. This is particularly important for simulating the multi-scale mechanical patterns of cardiac contraction and relaxation. Moreover, for multi-modal heart-on-a-chip platforms, systematic optimization and reporting of coupled stimulation parameters (amplitude, frequency, waveform, and phase across perfusion–deformation–pacing) remain essential to improve cross-platform reproducibility and physiological interpretability.

4.4 Tumor-on-a-chip

Tumors are abnormally proliferating multicellular tissues. They are characterized by highly heterogeneous cell populations and dynamically evolving microenvironments. Mechanically, tumor microenvironments exhibit specific physical properties. Matrix stiffness gradients typically range from approximately 1000 to 50,000 Pa. Interstitial fluid shear stress falls within the range of 0.01–0.2 Pa. Replicating these conditions requires tumor-on-a-chip systems to move beyond static culture paradigms [101]. A critical frontier for these platforms lies in delineating the stark mechanical divergence between malignant and healthy cell populations. While healthy interstitial tissues typically maintain low hydrostatic pressures (1000–5000 Pa) and homeostatic matrix compliance, the tumor microenvironment is characterized by a "mechanical paradox". Macroscopically, solid tumors exhibit a rigid bulk due to excessive ECM deposition and cross-linking, with matrix stiffness reaching gradients up to 50,000 Pa that drive epithelial-mesenchymal transition (EMT). Microscopically, however, individual metastatic cancer cells often display significantly lower cortical stiffness compared to healthy epithelial cells. This mechanical "softening" is a functional adaptation that facilitates their extreme deformation and "squeezing" through tight endothelial junctions during vascular extravasation. Furthermore, tumor cells exert markedly higher traction forces on the ECM to power collective invasion, whereas healthy cells utilize moderate adhesion forces primarily for structural stability and the prevention of anoikis. Modeling these mechanical disparities allows tumor-on-a-chip systems to transition from descriptive mimicry to predictive mechanobiological models. This integration is necessary to precisely replicate spatiotemporal heterogeneity in pressure, stiffness, and perfusion.

Such chips construct biomimetic microenvironments using 3D porous scaffolds (e.g., collagen/gelatin composite matrices with elastic moduli tunable to levels similar to tumor parenchyma), integrate miniature oxygen sensors and pH probes for real-time monitoring of metabolic heterogeneity, and employ gradient perfusion systems to simulate concentration gradients of key factors like vascular endothelial growth factor (VEGF). Figure 11(a) schematically summarizes how the extravasation-on-a-chip platform is engineered to reproduce the mechanical microenvironment that metastatic cancer cells encounter during vascular escape. Cancer cells are first grown as compact three-

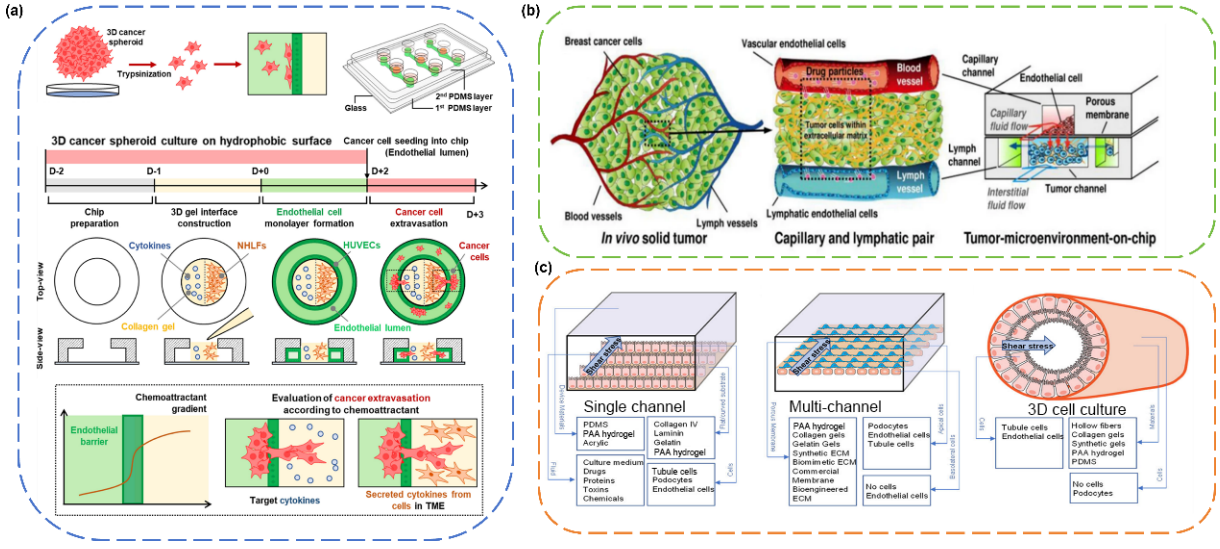

Figure 11 (a) Schematic illustration of the presented *in vitro* microfluidic model for cancer extravasation utilizing 3D cancer cell sources. Reproduced with permission from Ref. [103], © Lee, M. et al. 2023. (b) "Tumor-microenvironment-on-chip" (T-MOC) for NP transport assessment. Reproduced with permission from Ref. [19], © Tian, C. T. et al. 2022. (c) Shear stress applied to kidney-on-a-chip. Schematic drawing of the design for single-channel, multi-channel, and 3D cell culture devices. Reproduced with permission from Ref. [17], © Wang, D. et al. 2022.

dimensional spheroids on hydrophobic culture surfaces, which enhances cell–cell adhesion, internal solid stress and cytoskeletal tension while minimizing cell-substrate traction, thereby pre-conditioning a mechanically adapted, pro-metastatic population for subsequent loading into the chip. These cells are then introduced into a ring-shaped endothelial lumen surrounding a collagen-based stromal gel confined between stiff glass and PDMS walls, so that stromal-cell-driven gel contraction, endothelial tension and luminal fluid shear are all transmitted through a continuous gel–endothelium–fluid interface that defines realistic distributions of shear, compression and tensile stresses. Under controlled perfusion, the endothelial monolayer experiences physiologic shear stress that regulates junctional integrity and barrier mechanics, while a superimposed chemokine gradient and interstitial flow across the collagen generate pressure and shear gradients that bias cancer-cell polarization, traction organization and directed migration through the endothelial barrier into the three-dimensional matrix, thus enabling quantitative analysis of extravasation under well-defined, physiologically relevant mechanical cues.

In the early 2010s, researchers established foundational models using 3D ECM scaffolds and pressure-driven dynamic chambers. These models demonstrated a positive correlation between tumor cell migration direction and mechanical gradients. For example, one study utilized fiber-reinforced hydrogel composites. The team-controlled fiber density via electrospinning and the matrix modulus via photo-initiators. They observed that cells exhibited chain-like collective invasion guided by stiff fibers. In contrast, in low-stiffness matrices, they displayed amoeboid single-cell migration. This finding elucidated how tumor cells adjust their invasion strategy by sensing ECM stiffness differences [102]. However, this early-stage research had significant limitations. Static culture conditions could not simulate *in vivo* metabolic dynamics, such as hypoxia-induced ECM remodeling. Furthermore, the models lacked vascularized structures and immune interaction interfaces. The mid-2010s saw the evolution of microfluidic technology with multi-layer architectures. These systems featured distinct tumor-vessel-stroma zones. Through micro/nanofabrication techniques, they achieved multi-field coupling of blood flow shear stress and perivascular circumferential stress. This advancement successfully enabled the construction of vascular networks containing pulsatile flow (Fig. 11(b)). For example, in a 3D microfluidic chip designed by a team at Southeast University, HUVECs formed a monolayer in channels adjacent to a collagen matrix, simulating vascular wall mechanical load via periodic pressure drive; simultaneously, tumor cells (e.g., MCF-7) formed invasive aggregates within the 3D collagen gel, revealing the link between shear stress and tumor cell migratory behavior [103].

Entering the 2020s, the integration of multi-physics coupled systems became a new direction. Researchers combined flexible bio-electrode arrays with temperature control modules. This integration allowed for the synchronized collection of metabolomic and mechanical signals. It also verified phenomena like enhanced tumor cell membrane permeability using ultrasound fields. This technological advancement laid the foundation for more dynamic biomimetic systems. For instance, the synergistic application of ultrasound and electric fields allows programmable control over processes like tumor cell exosome release and immune cell migration pathways [104].

In summary, tumor-on-a-chip platforms have redefined cancer modeling. They enable the integration of mechanical, biochemical, and immune factors within controlled microenvironments. Nonetheless, they face persistent limitations. Standardization of mechanical inputs, such as shear amplitude and stiffness gradients, is often lacking. Cross-platform variability also hampers reproducibility. Furthermore, long-term metabolic coupling remains underexplored. Future efforts should focus on several key areas. One is establishing quantitative biomechanical benchmarks. Another is integrating adaptive sensing with AI-driven feedback control. A crucial step forward involves incorporating vascular-immune-metabolic tripartite coupling. This would better capture the dynamic reciprocity of tumor evolution. Only through such integrative and critical refinement can these systems transition from descriptive mimicry to predictive mechanobiological models. This evolution is essential for ultimately informing precision oncology.

4.5 Kidney-on-a-chip

The kidney is a highly heterogeneous organ. It comprises numerous functional cell types, including glomerular endothelial cells, podocytes, mesangial cells, and epithelial cells from different tubular segments. Together, these cells form an intricate blood filtration and reabsorption network. They collaboratively execute complex physiological functions. This functionality relies on a specialized microenvironmental regulatory system. The three-dimensional extracellular matrix scaffold provides essential mechanical support. It also co-regulates cell behavior through integrin-mediated mechano-transduction, growth factor gradients, and local fluid shear stress. Notably, kidney cells are persistently exposed to multi-modal mechanical stimuli. For instance, glomerular capillary endothelial cells endure pulsatile blood flow shear stress. This stress can reach levels up to 3–5 Pa. Meanwhile, proximal tubule epithelial cells experience periodic changes in luminal hydrostatic pressure, typically around 10–20 mmHg. Podocytes, on the other hand, sense mechanical tension across the filtration membrane via their specialized foot processes. These dynamic mechanical signals directly influence key cellular processes like proliferation, polarization, and barrier function.

Simple microfluidic channels offer an advancement over static culture by providing fluid conditions that better resemble *in vivo* dynamics. These chips simulate tubular flow by controlling key parameters like flow velocity and hydrostatic pressure. Typical velocities range from 0.1 to 1.0 mm/s. This flow produces a physiological shear stress of approximately 0.02 to 0.2 Pa. This level of stress is sufficient to influence cell adhesion, morphology, and transporter expression. For example, vascular endothelial growth factor modulates endothelial proliferation and differentiation under shear stimulation (Fig. 11(c)). Simultaneously, integrins regulate adhesion and migration through mechanosensitive feedback. By modulating luminal pressure, these models can also reproduce segmental hydrostatic variation and its effect on epithelial metabolism. Despite these advantages, a significant limitation remains. Their single-channel designs lack the spatial complexity needed to fully capture interactions between different nephron segments.

3D bioprinting technologies enable the creation of cell culture environments that closely mimic native kidney anatomy. By precisely arranging cells and biomaterials in three dimensions, these systems produce complex tissue constructs. Within these 3D structures, fluid flow generates shear stress that is more complex and physiologically realistic than in 2D models, better simulating the conditions found in renal tubules. Under such 3D mechanical

cues—including shear, tension, and compression—stress fibers and cytoskeletal proteins like actin and microtubules help regulate cell morphology, migration, and function. These bio-printed mechanical forces can be finely tuned to replicate natural physiological loading. Moreover, cell–cell interactions within 3D environments are more complex and varied, further shaping overall cellular behavior. While 3D bioprinting advances structural biomimicry, high-fidelity modeling necessitates transitioning from static architectures to dynamic, multi-functional integrated systems. To capture physiological complexity, these "organ-on-a-chip" platforms consolidate real-time electrochemical sensing, drug delivery, and multi-channel fluidics into interconnected modules. A primary advantage of this integration is the ability to monitor cellular metabolism and signaling *in situ*. For example, integrated electrochemical sensors can track real-time fluctuations in calcium ion channel activity, which regulates intracellular homeostasis in response to environmental stimuli. Beyond chemical signaling, the integration of physical sensors is equally indispensable for monitoring the kidney-on-a-chip's microenvironment. Since renal functions like ultrafiltration and reabsorption are driven by precise transmural pressure gradients (typically ranging from 1300 to 6700 Pa), on-chip pressure sensors allow for real-time tracking of luminal hydrostatic variations and can detect membrane clogging or flow instability. In addition, transepithelial electrical resistance (TEER) sensors or impedance-based electrodes can be integrated to continuously assess epithelial/endothelial barrier integrity under coupled shear-pressure stimulation, providing complementary functional readouts alongside biochemical assays. Furthermore, optical oxygen (or pH) sensors can be incorporated for *in situ* mapping of metabolic microenvironments, enabling correlation of mechanical loading with hypoxia/acidification-related functional changes. These integrated multi-modal sensing approaches provide a more holistic view of the kidney's mechanical and metabolic health. Finally, these platforms enable the synergistic application of mechanical forces—including shear stress, osmotic pressure, and solid mechanical strain—to simulate the renal microenvironment. This mechanical signaling is transduced via transmembrane receptors, such as G protein-coupled receptors (GPCRs), to modulate cellular responses.

By simultaneously controlling these mechanical inputs alongside precise chemical gradients, these systems provide a comprehensive, biomimetic framework for studying kidney physiology and pathology.

In summary, kidney-on-a-chip technology has evolved significantly. It has progressed from simple microfluidic channels to integrated, multi-functional, and high-throughput architectures. This evolution has markedly improved the mechanistic simulation of renal function. These systems demonstrate that mechanical forces are pivotal for reproducing renal filtration, reabsorption, and transport mechanisms when they are precisely regulated. However, persistent challenges remain. These include the lack of functional vascularization, limited integration of immune and metabolic modules, and a poor correlation between *in vitro* and *in vivo* mechanical thresholds. Future development should focus on several key areas. One is implementing real-time feedback-controlled mechanical actuation. Another is developing soft-material scaffolds that approximate the native elasticity of kidney tissue, which is typically in the 1000–5000 Pa range. Finally, AI-assisted data fusion could help unify mechanical, electrical, and biochemical readouts[105, 106]. Only through such standardization and closed-loop control can kidney-on-a-chip systems evolve. The goal is for them to become predictive, translational platforms useful for nephrotoxicity assessment and precision medicine.

4.6 Liver-on-a-chip

The liver is a highly mechanosensitive organ. Its performance in metabolism and detoxification is defined by a combination of factors. These include perfusion dynamics, matrix stiffness, and intercellular tension. In the living body, hepatocytes are continuously exposed to specific mechanical cues. They experience sinusoidal shear stresses of approximately 0.1 to 1 Pa. They also interact with a matrix that has an elastic modulus of 0.5 to 3 kPa. These biomechanical cues are not merely passive. They actively regulate crucial liver functions through mechano-transduction pathways. Key functions include bile secretion, protein synthesis, and cytochrome P450 enzyme activity. These processes are governed by signaling molecules such as integrins, YAP/TAZ, and calcium. Therefore, reconstructing these mechanical stimuli *in vitro* is a critical step.

Early liver-on-a-chip designs were relatively simple. They primarily focused on basic functions of monolayer hepatocytes. These functions included drug metabolism and detoxification. The initial models typically consisted of one or several cell channels. They offered only rudimentary fluid dynamic control. In recent years, this technology has undergone significant advancements. Modern chip designs now incorporate multi-layered cellular structures. These structures simulate the diverse cell types and microenvironment of the liver (Fig. 12(a)). The latest designs often include distinct layers. These are an endothelial cell layer, a hepatocyte layer, and a matrix layer. This approach more faithfully recapitulates the liver's structure and function. Furthermore, to enhance simulation accuracy, improved chips feature sophisticated fluid control systems. These systems enable more complex fluid dynamic simulations. For example, they can mimic blood flow and bile flow [107].

Within these systems, fluid shear stress is a key mechanical force. It is primarily implemented by simulating the effect of blood flow on endothelial cells. This shear stress modulates fluid velocity and pressure. The goal is to mimic the *in vivo* impact of blood flow on the liver (Fig. 12(b)). Research demonstrates the benefits of this approach. For instance, one study developed a perfusable micropillar array chip device. This system achieved high-throughput, controllable generation of hiPSC-derived embryoid bodies. It also promoted *in situ* differentiation of these bodies towards endoderm and hepatic lineages. This process leads to liver organoid formation under fluidic conditions. A significant advantage is the integration of these differentiation processes on a single chip. This integration simplifies the complex procedures of traditional organoid culture. Additionally, mechanical fluid shear stress was found to be beneficial. It promotes endoderm differentiation during liver development. This, in turn, encourages hepatic lineage specification. It also enhances liver-specific functions, such as albumin secretion and CYP450 enzyme activity. Enzymes of the Cytochrome P450 family are crucial for drug metabolism. Variations in fluid shear stress can influence their expression and activity. Consequently, this affects drug metabolic rates and detoxification functions. In liver-on-a-chip systems, mechanical stress simulates another aspect of the physical environment. It replicates cellular stretching and contraction experienced in native liver tissue. This mechanical stress

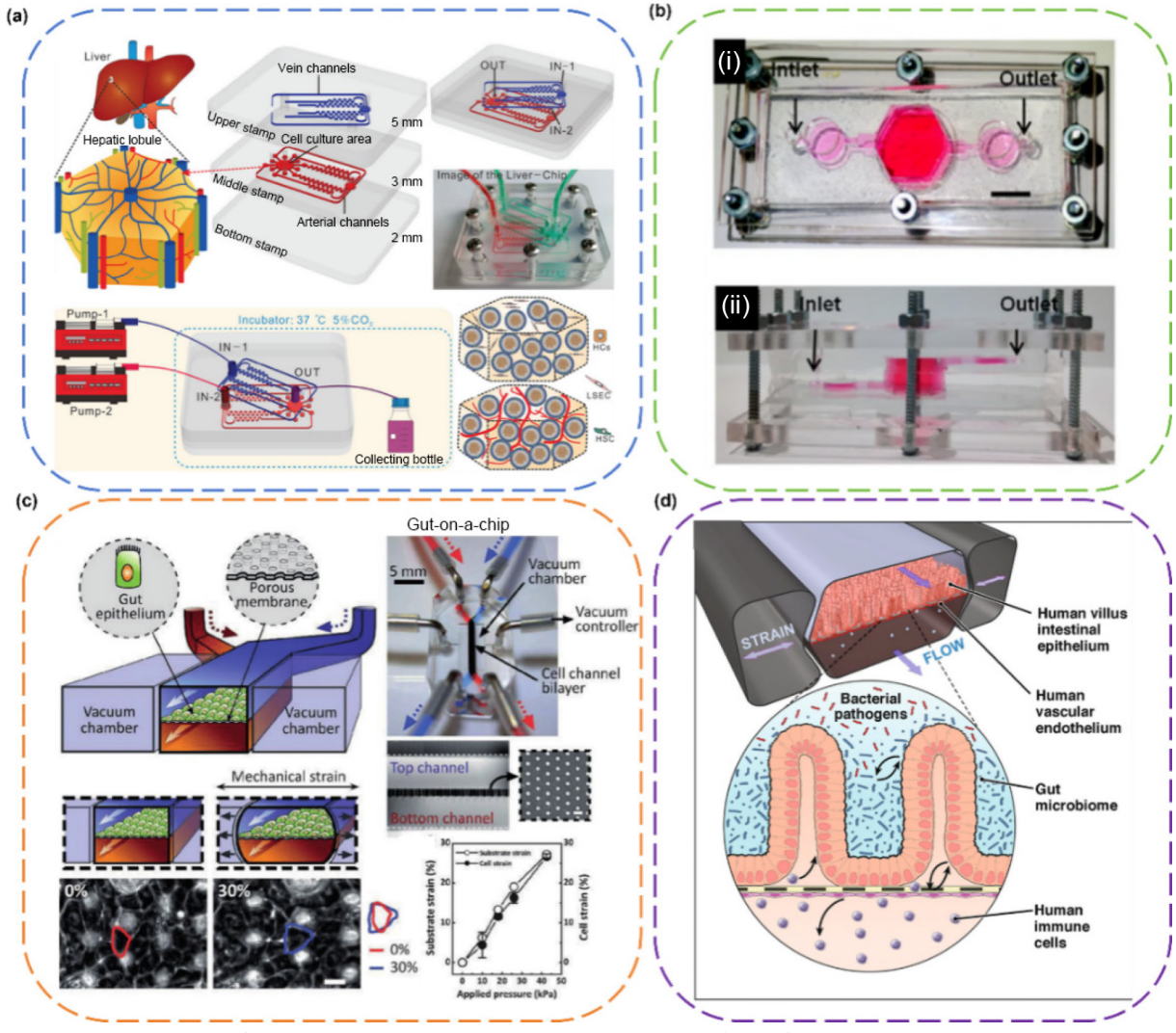

Figure 12 (a) Design and operation of the tri-vascular liver-on-a-chip. Reproduced with permission from Ref. [113], © Liu, J. et al. 2022. (b) Top-view (i) and side-view (ii) of the assembled liver-on-a-chip with the inlet and outlet fluidic ports as indicated. Reproduced with permission from Ref. [114], © IOP Publishing Ltd. 2016. (c) The human gut-on-a-chip. Reproduced with permission from Ref. [109], © The Royal Society of Chemistry 2012. (d) The mechanically active human Gut Chip. Reproduced with permission from Ref. [18], © Bein, A. et al. 2018.

significantly impacts hepatocyte function. For example, it can modulate intracellular fibronectin expression. This modulation occurs through alterations in the cell membrane's stress state. Fibronectin is a key component of the liver's extracellular matrix. It plays a critical role in cell adhesion, migration, and overall function. Mechanical stress can also regulate intracellular stress proteins. Heat shock proteins are one such example. These proteins respond to external mechanical stimuli.

Despite these advances, current liver-on-a-chip systems still face key mechanobiological limitations. A major challenge is the lack of perfusable vascular and immune components. These components are essential for mediating mechano-immunological communication in the body. *In vivo*, endothelial shear and sinusoidal pulsation have important regulatory roles. They regulate Kupffer-cell activity and metabolic zonation. However, such force-dependent feedback is largely absent in current chips. Integrating shear-responsive endothelial layers and mechanosensitive macrophages would help restore these interactions. This enhancement would significantly increase physiological relevance. Furthermore, the mechanical environment in most chips is homogeneous. This overlooks the native gradients of shear, pressure, and stiffness that define hepatic zonation. A promising direction is engineering spatially graded viscoelastic matrices. Combining these with programmable flow patterns could replicate these mechanical landscapes [108]. This approach would improve disease modeling and long-term functional fidelity.

In summary, future liver-on-a-chip platforms need to evolve. They should move beyond static reconstructions. The goal is to create adaptive, force-responsive systems. In these advanced systems, mechanical cues would actively regulate core hepatic processes. These processes include metabolism, regeneration, and pathology.

4.7 Gut-on-a-chip

Alterations in intestinal structure, mechanics, and physiology are fundamental to many acute and chronic intestinal diseases. Understanding gut health requires studying the coordinated regulation of its dynamic three-dimensional structural remodeling. This includes the formation of crypt-villus units. Another critical aspect is its mechanical environment, which involves periodic

peristaltic stress. Furthermore, its multi-interface physiological functions, such as nutrient absorption, barrier defense, and immune modulation, are essential. Consequently, a key direction in advancing intestinal research is the development of gut-on-a-chip systems with comparable biomimetic capabilities.

Gut-on-a-chip systems are specifically designed to simulate the complex environment of the intestine. They replicate the structure of the intestinal epithelium, interactions with the gut microbiota, and intestinal mechanical and fluid dynamics (Fig. 12(c)). The earliest gut-on-a-chip model consisted of a device with two parallel microchannels separated by a flexible, porous (5–7 μm pore diameter) membrane coated with ECM. One side of the membrane was lined with cultured human Caco-2 intestinal epithelial cells, and culture medium was perfused through both channels (Fig. 12(d)). A low fluid shear stress, typically 0.02 dyne/cm^2, was generated by flowing fluid at a slow rate of 30 μL/h. The luminal microenvironment was simulated by applying cyclic mechanical deformation to the membrane and its attached cell layer. This deformation mimicked peristalsis-like motions and was achieved by using vacuum actuation of hollow side chambers within the flexible device [109, 110].

With ongoing technological advancements, gut-on-a-chip designs have become more sophisticated. They now progressively incorporate more complex multi-layer structures. These structures include matrix layers, endothelial interfaces, and advanced peristaltic actuation systems. The goal is to reproduce gut physiology with greater fidelity. In these advanced systems, luminal shear stress is carefully controlled within a range of 0.02 to 0.2 dyn/cm^2. This reproduces the effect of chyme flow and influences epithelial cell alignment and the expression of tight-junction proteins like cadherins and occludin. The systems also introduce rhythmic tension and compression to mimic intestinal peristalsis. This mechanical stimulation helps regulate cell differentiation and mucus secretion. Importantly, these biomechanical stresses activate specific signaling pathways, including YAP/TAZ and MAPK cascades. These pathways link the biomechanical stress to epithelial turnover. Furthermore, the chips emulate chemical gradients found in the gut. These gradients are crucial for simulating nutrient absorption and xenobiotic metabolism. They modulate enzymatic activities such as those of intestinal esterases and CYP3A4 [111]. However, a significant limitation of most current systems is their reliance on uniform, cyclic stretching. This approach fails to capture the propagating peristaltic waves that drive directional transport and mix microbiota in the living intestine.

In summary, gut-on-a-chip technology has become a powerful platform for research. It is used to dissect intestinal physiology, disease mechanisms, and host-microbe interactions [112]. Nonetheless, its maturity for translational applications is constrained by several core challenges. The first challenge is establishing a long-term co-culture of intestinal epithelia with complex, often anaerobic, microbiota under stable oxygen gradients. The second involves integrating essential immune and neural components to recapitulate the gut-brain-immune axes. The third challenge is replicating the biomechanical heterogeneity of the gut through multi-frequency, propagating peristaltic actuation, moving beyond simple uniform deformation. Moreover, recreating a self-renewing mucus layer with a physiologically relevant thickness of 100 to 300 micrometers and appropriate viscoelasticity remains a critical unmet goal. Finally, most current models focus only on epithelial monolayers. They fail to encompass the full-thickness architecture of the intestine, which includes layers like the submucosa, muscularis externa, and the enteric nervous system.

Future efforts should prioritize the development of closed-loop micro-physiological systems. These systems would be capable of dynamically adjusting flow, strain, and oxygenation in real time. This capability should be supported by integrated biosensors that provide mechanical and metabolic feedback. The next generation of gut-on-a-chip platforms can evolve from static mimetic systems into predictive mechanobiological tools. This will be achieved by coupling epithelial, microbial, and immune modules under controlled mechanics. Such advancements will reveal how biomechanical and microbial cues jointly orchestrate gut homeostasis and disease.

5 Challenges and opportunities

As powerful life science and engineering methodologies, novel concepts and approaches related to organs-on-chips have grown exponentially over the past few years. With the rapid development of OoC technology, mechanical control has become a core element in constructing high-fidelity physiological models. Current research has surpassed the limitations of static culture. Through the integration of technologies such as microfluidics, flexible sensing, and dynamic stress loading, precise simulation of multi-modal mechanical stimuli—including shear stress, compressive force, and tensile stress—has been achieved. Representative accomplishments, such as the replication of dynamic deformation during respiratory motion in lung-on-a-chip systems and the elucidation of endothelial cell responses to blood flow shear in vascular chips, signify that mechanics-driven OoC technology has entered a new phase of functional biomimicry. However, several challenging issues still need to be addressed. Alongside these challenges, there are promising opportunities awaiting exploration. Based on experience, review, and reflection, we can elaborate on the challenges and opportunities in mechanics-regulated organs-on-chips. These can be categorized into four main areas. The first area is multi-scale mechanical integration. The second is the sustained precision of mechanical stimulation. The third concerns the functionality of bio-interface materials. The fourth area involves cross-organ interactions (Fig. 13).

OoC systems need to simultaneously simulate multi-level mechanical environments, ranging from intercellular forces to tissue-level stresses, yet existing technologies are often limited to single-scale control. For instance, while microfluidic systems can precisely control fluid shear stress, they struggle to concurrently apply dynamic compressive forces at the cytoskeletal level. Similarly, programmable actuators can simulate periodic stretching but cannot replicate the nanoscale mechanical gradients at focal adhesions. This fragmentation hinders the models' ability to truly reflect the spatiotemporal coupling characteristics of *in vivo* mechanical signals. Future efforts should focus on developing multi-modal integrated platforms that combine micropillar arrays, variable stiffness substrates, and dynamic flow field control to achieve cross-scale synergistic mechanical loading. Predicting the interactive effects of mechanical parameters using deep learning algorithms could potentially establish a unified modeling framework, spanning from molecular mechano-transduction to organ morphogenesis.

Significant deficiencies persist in the sustained precise control of

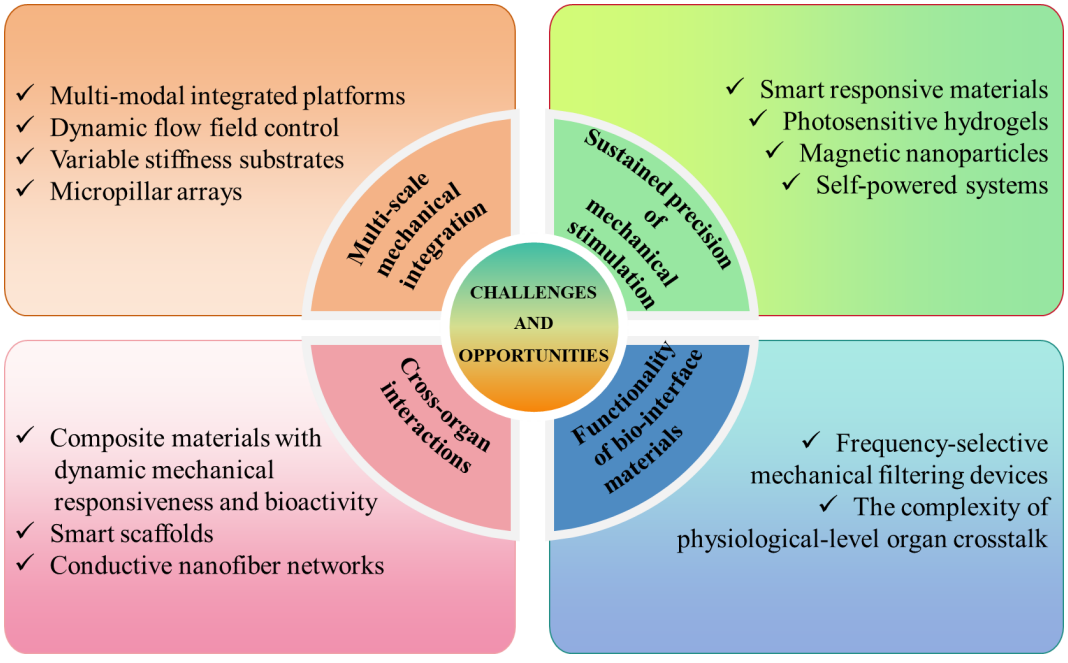

Figure 13 The demonstration of challenges and opportunities of the OoC.

mechanical stimulation in current systems. Long-term operation of micropumps is susceptible to interference from bubbles or particle clogging, leading to fluctuations in shear stress. The mechanical properties of PDMS substrates cannot be dynamically adjusted after curing, making it difficult to simulate the evolution of tissue stiffness during development. The development of smart responsive materials may be key to breaking this impasse, for example, photosensitive hydrogels allow real-time adjustment of elastic modulus via light exposure, and magnetic nanoparticles enable remote manipulation of local stress distribution. Furthermore, the design of self-powered systems (e.g., utilizing metabolic byproducts from organoids to drive microfluidics) holds promise for enhancing the long-term operational stability of devices, providing support for the construction of chronic disease models.

Current supporting materials face challenges in balancing mechanical compatibility with biological functionality. Although PDMS offers excellent optical transparency, its hydrophobic surface requires chemical modification to promote cell adhesion, and its static stiffness cannot mimic the dynamic remodeling of the ECM. Natural hydrogels (such as matrigel), while providing a biomimetic microenvironment, suffer from low mechanical strength and significant batch-to-batch variation. Developing composite materials that possess both dynamic mechanical responsiveness and bioactivity is an important direction. For instance, co-crosslinking thermo-sensitive polymers with ECM proteins can create smart scaffolds whose stiffness changes with temperature gradients. Incorporating conductive nanofiber networks can both transmit mechanical forces and monitor electrophysiological signals, offering new tools for mechano-electrical coupling research.

The interconnection of multi-organ chips faces the challenge of mechanical coupling distortion. Mechanical signals between human organs (e.g., blood flow driven by cardiac contraction, thoracic pressure changes generated by lung respiration) are transmitted through the circulatory system and tissues, forming a dynamic network. However, existing chip models struggle to replicate this real-time mechanical coupling between multiple organs. For example, the contraction of a heart chip might influence the fluid shear stress in a connected liver chip, but the dynamic feedback mechanism of this cross-organ mechanical response has not yet been effectively implemented. When blood flow shear stress and respiratory motion are superimposed in microfluidic channels, pressure waveform distortion at the interface can significantly affect the predictive accuracy of pharmacokinetics. Current solutions often employ simplified fluidic networks, which fail to replicate the complexity of physiological-level organ crosstalk. Utilizing frequency-selective mechanical filtering devices (such as tunable micro-cavity arrays) to directionally transmit mechanical waves of specific frequencies might enable the reconstruction of inter-organ mechanical communication.

Standing at a technological inflection point, mechano-responsive organs-on-chips are evolving from discrete functional replication towards system-level physiological reconstruction. Future breakthroughs will depend on the deep integration of materials science, micro/nanofabrication, and computational biology. Hydrogels capable of adapting to the mechanical environment, biomimetic interfaces with self-sensing capabilities, and intelligent microfluidic networks supporting multi-organ coupling may redefine the paradigm for constructing *in vitro* models. As the precision of mechanical control approaches the subcellular scale and temporal resolution reaches the millisecond range, humanity may be poised to decode the mechanical codes of life on a chip, opening entirely new dimensions for disease mechanism research, personalized therapy, and novel drug development. This exploratory path requires not only technological innovation but also a return to biological essence, seeking the fundamental logic of life's design within the symphony of mechanics and biochemistry.

6 Conclusion

This review has systematically examined the crucial role and application progress of mechanical forces in organoid culture and OoC technology. At the fundamental theoretical level, it elucidated the perception and transduction mechanisms of mechanical signals

such as pressure, fluid shear stress, adhesion forces, and cell contractility, revealing the regulatory principles governing how the mechanical microenvironment influences cell behavior, tissue morphology, and functional expression. At the technological methodology level, this review provided a comparative analysis of the mechanical action modes in systems with solid substrates versus non-contact dynamic culture systems. It explored how innovative technologies like microfluidics, bioprinting, and magnetic levitation optimize the physiological relevance of organoids through precise mechanical control. Further focusing on typical case studies involving brain, lung, tumor, and kidney OoC systems, it showcased the core value of mechanical simulation in areas such as vascular network construction, replication of respiratory motion, regulation of tumor invasion, and biomimicry of renal filtration function.

Although organs-on-chips have demonstrated immense potential in disease modeling, drug screening, and personalized medicine, their development still faces multiple challenges: Insufficient precision in the dynamic integration of mechanical parameters limits the simulation of microenvironment heterogeneity; the balance between long-term mechanical stability and functional maintenance during extended culture has yet to be achieved; the mechanisms of mechanical coupling in multi-organ interconnected systems remain to be fully elucidated; and technological bottlenecks concerning standardization and large-scale production for clinical translation urgently need addressing. Future development should focus on the following dimensions: optimizing multi-scale mechanical parameter co-regulation by integrating AI algorithms; developing smart responsive materials to achieve dynamic adaptation of the mechanical microenvironment; and constructing inter-organ mechanical interaction interfaces to simulate systemic pathophysiological processes.

Prospectively, with the deep integration of mechanobiology, micro/nanofabrication, and biosensing technologies, OoC systems are expected to transcend current technological boundaries, opening new paradigms in understanding organ development mechanisms, modeling complex diseases, and advancing regenerative medicine. Innovations in novel biomimetic materials, flexible electronic devices, and adaptive control systems will not only enhance the physiological fidelity of organoid models but may also catalyze disruptive technologies such as “digital twin organs”. We firmly believe that mechanical control, as a core logic in OoC design, will continue to drive the paradigm shift in biomedical research from static replication towards dynamic biomimicry, providing revolutionary tools for humanity to explore the mysteries of life and conquer major diseases.

Data availability

Not applicable.

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Declaration of competing interest

All the contributing authors report no conflict of interests in this work.

Author contribution statement

Y.-J. L.: Conceptualization, data curation, formal analysis, investigation, methodology, writing – original draft, writing – review & editing. Y.-Y. Z.: Conceptualization, data curation, formal analysis, investigation, methodology, writing – original draft, writing – review & editing. W. W.: Conceptualization, data curation, funding acquisition, investigation, methodology, project administration, supervision, validation, writing – original draft, writing – review & editing. C.-S. Y.: Data curation, investigation, methodology, software. Y. Z.: Conceptualization, data curation, funding acquisition, investigation, supervision, validation, writing – original draft, writing – review & editing. Q. Z.: Conceptualization, supervision, validation, writing – original draft, writing – review & editing.

Use of AI statement

None.

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