Journal of Translational Medicine

REVIEW

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Advances in humanoid organoid-based research on inter-organ communications during cardiac organogenesis and cardiovascular diseases

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Abstract

The intimate correlation between cardiovascular diseases and other organ pathologies, such as metabolic and kidney diseases, underscores the intricate interactions among these organs. Understanding inter-organ communications is crucial for developing more precise drugs and effective treatments for systemic diseases. While animal models have traditionally been pivotal in studying these interactions, human-induced pluripotent stem cells (hiPSCs) offer distinct advantages when constructing in vitro models. Beyond the conventional two-dimensional co-culture model, hiPSC-derived humanoid organoids have emerged as a substantial advancement, capable of replicating essential structural and functional attributes of internal organs in vitro. This breakthrough has spurred the development of multilineage organoids, assembloids, and organoids-on-a-chip technologies, which allow for enhanced physiological relevance. These technologies have shown great potential for mimicking coordinated organogenesis, exploring disease pathogenesis, and facilitating drug discovery. As the central organ of the cardiovascular system, the heart serves as the focal point of an extensively studied network of interactions. This review focuses on the advancements and challenges of hiPSC-derived humanoid organoids in studying interactions between the heart and other organs, presenting a comprehensive exploration of this cutting-edge approach in systemic disease research.

Keywords humanoid organoids, heart-organ communication, cardiac organogenesis, cardiovascular diseases, omics technologies

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Introduction

The progression of cardiovascular diseases (CVDs) involves significant functional and structural alterations in vital organs and tissues [1]. Under stress and pathological conditions, the body regulates the balance of glomerular filtration rate and blood pressure through sympathetic stimulation, culminating in interaction relationships within the heart-brain-kidney axis [2]. The inflammatory response induced under pathological conditions plays a crucial role in inter-organ communication through cytokine secretion. This leads to interconnected communication between the heart and lungs [3], as well as between the heart and liver tissues [4]. Additionally, alterations in gastrointestinal microbiota [5] and neurologic damage in the brain [6, 7] have been reported to be strongly associated with the onset and progression of CVDs. Understanding the mechanisms of inter-organ communications is imperative for developing more precise drugs and effective treatments for CVDs and related organ pathologies.

Animal models are valuable tools for studying complex inter-organ communication, particularly in allergic and other non-infectious systemic diseases characterized by multifactorial effects [8]. As a significant relay organ, the spleen has also been shown to reduce infarct size in animal models by releasing cardioprotective factors during vagal activation [9]. Despite their effectiveness, animal models have some limitations, including significant interspecies differences in genetic expression and environmental factors, which hinder the replication of human conditions. Critically, patterns of gene expression regulation, inflammatory responses, and metabolic activity differ significantly between animal and human hearts [10]. To overcome these limitations and more realistically simulate physiological and pathological mechanisms, there is an urgent need for in vitro humanoid organoid or tissue models.

The discovery and development of human pluripotent stem cells, including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), has ushered in a new era of transformative research into the specific development of human organs and diseases [11]. Notably, the distinctive capability of hiPSCs to faithfully replicate a patient's genetic background makes them an invaluable in vitro platform for studying the effects of critical cell-cell interactions (CCIs) and inter-organ communications in pathological situations, thereby enhancing the relevance of findings to patient-specific conditions [12]. hiPSCs can differentiate into specific organ-specific cells, allowing for the investigation of organ interactions through two-dimensional (2D) cell co-culture [13]. With the continuous advancements in biotechnology and tissue engineering, recent developments in three-dimensional (3D) organoid technologies, such as multilineage organoids, assembloids, and organoids-on-a-chip, which provide enhanced structural and functional fidelity, offer great potential for constructing functional humanoid organoids from hiPSCs. These advancements facilitate the study of inter-organ communications during organism development and disease progression [14]. Furthermore, emerging omics technologies such as single-cell and spatial transcriptomics enable more sophisticated analysis of these heterogeneous and complex tissues [15].

This review focuses on developing and applying hiPSC-derived 2D and 3D models for investigating heart-organ interactions under both physiological and pathological conditions. Additionally, it explores emerging omics techniques used in the study of inter-organ communications.

Application of 2D co-culture models in the study of CCIs

Understanding the cardiovascular system requires a multilevel and multifaceted approach that encompasses multiple organs and cell types. 2D co-culture models based on the hiPSCs can build bridges between different specific cells and investigate potential interaction effects [16, 17]. In the cardiovascular field, the current 2D co-culture model is mainly concerned with the interaction effects of cardiomyocytes with the neural-immune microenvironment, which are crucial for understanding cardiac function and pathology.

While the innervation of the autonomic nervous system in the heart is critical in the cardiac pathophysiological, the neurocardiac co-culture model provides an essential method for exploring the signaling effects of the heart in response to external stimuli, and for studying cardiac arrhythmias, neuro-cardiotoxicity, and safety assessment [18, 19]. The roles of both the sympathetic and parasympathetic nervous systems in the heart have been studied using the neurocardiac co-culture model. Yoh-Suke Mukouyama and colleagues developed a direct co-culture model of sympathetic neurons with cardiomyocytes, demonstrating that the presence of sympathetic neuron upregulated contractile function-related genes in cardiomyocytes [20], such as those encoding fine myofibrillar filaments and thick myofibrillar filaments, as well as genes related to voltage-dependent Na^+, and inwardly rectifying K^+ ion channels. Structurally, sympathetic neurons also promoted the formation of gap junctions in cardiomyocytes via connexin 43 (CX43), facilitating intercellular contact and allowing action potential propagation. To explore whether the parasympathetic nervous system can establish functional connections with target tissues, Nadja Zeltner et al. constructed a direct co-culture model of cardiomyocytes and parasympathetic neurons [21]. This model revealed a reduction

in cardiomyocyte beating in response to acetylcholine receptor stimulation on parasympathetic neurons. Additionally, exposure to inflammatory factors such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), in combination with indirect co-culture in parasympathetic neuron medium, significantly restored reactive oxygen species (ROS) levels and beating variability in pathological cardiomyocytes, bringing them closer to the levels observed in healthy cardiomyocytes. These findings underscore the dual regulatory role of the nervous system in cardiac tissue, with the exact mechanisms still warranting further investigation.

The functional role of macrophages in the microenvironment of the infarcted heart, especially their interactions with cardiovascular cells, remains an area of ongoing investigation [22]. In response, the immune-cardiac co-culture model has emerged. hiPSC-derived allogeneic macrophages significantly improved cardiac function by injection into the infarcted myocardium of rats, mainly as a result of the exchange of damaged macrophages with healthy ones [23, 24]. Macrophages exchange sodium, potassium, and calcium ions through gap junction protein 43, either directly or indirectly [25]. In co-cultures of cardiac macrophages and iPSC-CMs, direct membrane contacts are formed, exerting electrophysiological modulation on the contractility of cardiomyocytes [26]. These findings suggest that the immune-cardiac co-culture model is a promising platform for studying ion channel diseases such as arrhythmias. By modulating macrophage states in inflammation, fibrosis, tissue regeneration, and repair, this model can also provide insight into therapeutic strategies for adult myocardial infarction [27, 28].

In addition to macrophages, other immune cells also play a significant role in maintaining cardiac homeostasis. By using the direct co-culture model, Kunal Sikder and colleagues investigated the mechanism of leukocyte-cardiomyocyte crosstalk [29]. They found that elevated levels of interleukin-1 beta (IL-1β) and TNF-α induce leukocyte recruitment to the injured tissues, where increased expression of alpha-smooth muscle actin (αSMA) and intercellular cell adhesion molecule-1 (ICAM-1) indicates leukocyte activation and migration. Furthermore, IL-1β and TNF-α enhance chemokine chemotaxis to the injured area via the danger-associated molecular patterns (DAMP) signaling pathway, disrupting cardiac homeostasis and contributing to the progression of chronic heart failure through cardiomyocyte loss.

Preadipocytes also secrete large amounts of pro-inflammatory cytokines [30]. Ichiro Morioka et al. explored the interaction between epicardial adipose tissue and myocardial tissue using an indirect co-cultured model [31]. They found that human adipose-derived dedifferentiated adipose cells secret various pro-inflammatory mediators, such as chemokine ligands 1 and 12, granulocyte colony-stimulating factor, IL-6 and IL-8, macrophage migration inhibitory factor This led to decreased cardiomyocyte function, as evidenced by reduced contraction frequency and increased oxygen consumption rates, contributing to a better understanding of the pathogenesis of epicardium-associated heart failure.

The heart and vascular network are the two most critical components of the cardiovascular system. Current reports highlight the concern of heart failure with preserved ejection fraction, particularly in women, suggesting that postmenopausal estrogen deficiency may be involved in the pathogenesis. Eiki Takimoto and colleagues explored the crosstalk between endothelial cells and cardiomyocytes using a direct co-culture model to uncover the mechanisms underlying estrogen deficiency-associated heart failure with preserved ejection fraction [32]. They demonstrated a pivotal role of the endothelial estrogen-myocardial cGMP (cyclic guanosine monophosphate) axis in angiogenic response and cardiac functional benefits, providing mechanistic insights into our understanding of sex differences in cGMP-related treatments for heart failure.

Despite the advantages of 2D culture models, such as ease of cultivation for high-throughput screening, they remain monotypic and lack the complex, self-contained matrix microenvironment inherent in 3D structures, which is essential for a nuanced interpretation of CCIs [33]. Meanwhile, current 2D cell-based in vitro disease models are limited in generalizing inter-organ communications [34]. Recent advancements in emerging technologies, including multilineage organoids, assembloids, and organoids-on-a-chip, have significantly improved our understanding of complex biological interactions, such as inter-organ communications.

Humanoid organoid models for studying inter-organ communications

Organogenesis is a coordinated process involving interactions among different embryonic tissues. 3D culture allows for the generation of in vitro tissues with organ functions similar to primary tissues [35]. Compared to the 2D cellular level, 3D tissue models are more effective in eliciting cellular fates resembling those observed in vivo during specific processes [36]. These organoids can effectively reproduce the complex structure of living organs and inherit the genetic background of the donor, making them invaluable tools for disease modeling, unravelling pathophysiological mechanisms, and elucidating developmental biology [37]. Research on cardiovascular organoids has advanced into various areas, including myocardium, blood vessels, and valves, providing numerous models for studying CVDs [38].

Combined with tissue engineering techniques, organoids-on-a-chip based on microfluidic devices have been developed [39]. It is now possible to assemble more complex tissue-assembled bionic organoids, which provide near-realistic models for studying human tissues and organs, as well as for simulating pathological processes [40]. Humanoid organoid models facilitate macroscopic and microscopic analyses of intercellular crosstalk, resulting in multi-organ models that effectively simulate complex tissue-tissue and inter-organ communications, making them suitable for organ development, toxicity, and efficacy [41]. This section describes the three main humanoid organoid models for studying inter-organ communications.

hiPSC-derived multilineage organoids

Most cardiac organoid studies have focused on individual organ genealogies or specific signaling pathways [42]. Although they show great potential in recapitulating the physiology of the human heart, they fail to capture the complex crosstalk between the heart and endodermal or ectodermal organs, limiting our understanding of integrated organ development [43]. Consequently, we still lack a comprehensive understanding of the development of multi-organ genealogies. Currently, organoids can self-assemble into ordered patterns and structures and generate multilineage organoids, a process guided by hiPSC aggregates and bi-directional Wnt signaling, which is crucial for mimicking developmental processes [44]. Moreover, recent reports indicate that hiPSC-derived multilineage organoids composed of cardiac and other tissues can reflect the structural and early developmental patterns of the embryonic heart, providing insights into the co-developmental relationship of cardiac development with foregut endoderm and ectoderm [45, 46]. Retaining cellular complexity, these organoids reveal critical structural features of the human heart and gut development.

Multilineage organoids provide an unprecedented opportunity for understanding organ development and shedding light on how the endoderm and heart cooperate to guide morphogenesis, patterning, and maturation. Specifically, these organoids recapitulate key morphogenetic events that occur primarily after heart tube formation and preserve these tissues and functions for up to 100 days [47]. Furthermore, multilineage organoids provide a microenvironment that permits the formation of intestinal tissue while promoting the maturation and stabilization of cardiomyocytes. Compared to animal models, multilineage organs provide a more direct view of tissue crosstalk mechanisms. As such, they represent a promising avenue for generating more physiologically relevant in vitro models of human development.

However, the reproducibility of multilineage organoids from each batch is a challenge due to the large size, high cell density, and inherent heterogeneity of multilineage organs. Additionally, the dynamic and complex processes within organoids necessitate collecting different batches of organoids independently, making the process time-consuming. The experimental variability of multilineage organoids, primarily stemming from the lack of external guidance for multilineage organoids and their reliance on self-organized structural reconstruction, results in unclear and dynamically unstable organoid compositions as they mature. The developmental patterns of different lineage vary across organs, highlighting the need for further clarification of the intersections between the developmental trajectories of multilineage organoids. Moreover, multilineage organoids still fall short of fully replicating the structure of mature organs, lacking the microenvironment and critical cellular interactions within the tissues. To address these limitations, efforts must focus on constructing organoids that better emulate the human microenvironment.

Assembloids

The sophistication of organoids is continually increasing to reflect the complexity of the human body's multiple tissues. However, the cellular interaction network of multilineage organoids is not sufficient to form complex tissue microenvironments and lacks the required spatial organization [48, 49]. To address this limitation, assembloids composed of multiple organoids have been developed [50, 51]. Developed by Sergiu Paca's group, assembloids represent an advanced extension of the 3D multicellular system [52]. Assembloids are created by combining two or more distinctly patterned organoids, integrating additional cell or tissue types. Assembloids are invaluable tools for modeling complex cellular integration within and between tissues, providing insights into the pathophysiology of various diseases [53].

Due to the lack of innervation and vascular network, cardiac organoids are unable to fully replicate the in vivo environment required for organ development, integrity, and functional coupling. To model organ complexity and heterogeneity, cardiac assembloids consisting of atria, atrioventricular canal cardiomyocytes, and ventricular globules have been developed to mimic the atrial-ventricular interface [54]. These models are particularly useful for studying complex cardiac conduction disorders, such as atrioventricular block, by reproducing the electrical propagation patterns seen in the heart. Moreover, cardiac organoids can be innervated with sympathetic neurons to construct neuro-cardiac assembloids, facilitating the modeling of inter-organ communications [55]. Anna F Rockel et al. developed a neuro-mesoderm assembly model, in which the co-developing of mesodermal and

neuroectodermal tissues and the formation of a vascular plexus along with the peripheral nervous system was observed, allowing for the study of crosstalk between neuroectoderm and mesoderm, and between peripheral neurons/neuroblasts and endothelial cells [56].

Our group developed a methodology for generating vascularized and chamber-like cardiac organoids by enveloping hiPSC-derived aggregates destined for vascular fate with the differentiated cardiomyocytes. This configuration allows vascular-committed cells to migrate outward in response to a vascular endothelial growth factor (VEGF) gradient, thus vascularizing the peripheral myocardium [57]. This model facilitated the discovery of interactions between endothelial cells and cardiomyocytes through the PI3K-AKT signaling pathway, extracellular matrix (ECM) receptor interactions, and Ras signaling pathway. Notably, a cluster of neurocytes was identified in these cardiac assembloids, providing a unique platform for studying neuro-cardiac interactions in the future.

In order to investigate the critical cardio-pulmonary mutual interaction during human embryogenesis, Ng et al. developed a cardio-pulmonary assembly model by the 3D suspension culture of co-induced cardiac and pulmonary progenitors from hiPSCs [58]. While the cardio-pulmonary assembly is initially arranged in the pulmonary-centered, concentric manner, the heart and lung compartments gradually move away from each other, eventually separating from each other, mimicking the distinct tissue development seen in the body. Therefore, these assembloids provide a powerful tool for observing organ interactions, though further investigation is needed to understand the specific regulatory mechanism involved.

In addition to cardiac assembloids, brain organoid assembloids containing multiple brain regions have been developed for elucidating and treating neurodevelopmental disorders [59, 60]. Based on these assembloids, studies have explored interactions between the nervous and immune systems [61], nerve-muscle organoids [62], and functional vascular brain assembloids consisting of human cortical organoids and the vascular system [63]. With advances in 3D bioprinting technology, which allows for precise control over the size, shape, structure, and organization of the printed tissues, more robust and scalable assembloid systems can be developed [61]. Despite these advancements, a human heart-brain assembloid has yet to be developed or tested, particularly using iPSCs derived from patients with pathological dysfunctions in both heart and brain tissues, such as those seen in Dravet syndrome caused by haploinsufficiency of the sodium voltage-gated channel alpha subunit 1 (SCN1A) gene [64]. These models can provide valuable insight into the role of target genes in the heart-brain axis.

The complexity of assembloids depends on two key factors, the generation of specific cell types and their diversity, which result in significant heterogeneity in assembloid components [65]. Moreover, it's important to note that the culture standards for assembloids have not yet been fully standardized. Assembloids lack precise techniques to quantify various aspects of pathophysiology. More refined and complex microfluidic technologies are needed to serve as a platform for the mass production of subsequent models. Combining these advancements with emerging omics technology will enhance the relevance of experimental results to personalized clinical patients, significantly improving the feasibility of clinical translation.

Organoids-on-a-chip

To simulate more complex microenvironments of multi-organ systems, current 3D tissue construction techniques combined with tissue engineering enable the creation of environments more compatible with human systems [66]. Organoids-on-a-chip refers to the integration of multiple organoid systems in a co-culture system, achieved by linking various organoid models within a microfluidic device [67]. This setup facilitates the co-culture of different types of organoids in a regionalized microenvironment. Specially, hiPSC-derived organoids-on-a-chip technology offers the advantage of incorporating the complete polygenic disease background of the patient [68]. This approach not only avoids ethical concerns but also leverages CRISPR/Cas9 technology to modify gene interactions, allowing for the modeling of complex diseases.

As a research platform that incorporates cellular and organoid models, organoids-on-a-chip effectively recaptures the communication between connected organs [69]. Organs-on-a-chip supports inter-organ communications by precisely controlling chemical, physical, and cellular micro-environments. This modular platform could effectively mimic barrier effects and mechanical interactions between different organ tissues [70]. Additionally, organoids-on-a-chip demonstrates reproducibility, including tumor structure, heterogeneity, function, and genetic stability in culture [71]. These systems also allow real-time monitoring of cellular responses to various stimuli, revealing disease mechanisms and their role in signaling pathways.

Organoids-on-a-chip has been used to study the interactions of multiple organ axis. For example, studies have focused on the interactions of intestinal tissue with other organs. This includes investigating phenomena such as the barrier effects of the gut-brain axis, the metabolic dynamics of the gut-hepatic axis, drug absorption, and toxicity assessment through the intestinal-renal axis, the telecommunications effects of the gut-lung axis, and

the immune interactions of the enteropancreatic axis [72]. Furthermore, the complex network of biochemical signals and mechanical forces in the bone-organ axis has been applied to explain mechanisms related to bone disease, drug screening, and pharmacokinetic analysis. These platforms have also been utilized to model complex physiological and pathological responses [73]. Based on these applications, the interactions between the heart and other organs in organoids-on-a-chip will be discussed in the following sections.

Despite the advancements, the application of microfluidic organoid chips faces three main challenges. First, the interactions between the microfluidic device environment and the cells, as well as between the cells within the device, remain underexplored. Emerging artificial intelligence (AI) techniques, such as large language models and AI-based analysis, may provide potential solutions for these challenges. Secondly, organoid culture processes lack a complex cellular microenvironment and rely on animal-derived matrices, such as substrates, whose composition is not well defined. Long-term culture and viability remain potential challenges. Lastly, the lack of standardized culture protocols is a significant limitation. Variations in patient samples, culture methods, media choices, and the absence of standards for organoid maturity all impact the consistency and application of organoids-on-a-chip models in clinical translational research.

Application of humanoid organoid models

Humanoid organoid models have great potential for simulating organogenesis, disease modeling, and drug screening at the multi-organ systems level, with the ultimate goal of playing a pivotal role in personalized medicine research. In order to understand the mechanisms governing interactions between the heart and multiple other organs in the organism, more targeted and effective therapeutic strategies must be developed. Based on the above models, communications between the heart and other organs have emerged as a significant field of study, which will be primarily discussed in the following context (Fig. 1), and summarized in Table 1.

Heart-organ axis-based organogenesis

Endodermal, mesodermal, and ectodermal structures can differentiate into specific tissues and organs. Some findings indicate defects in the thyroid gland and heart when the interplay between the germ layers is disturbed [74], underscoring the importance of germ-layer communications. Recent single-cell transcriptomics is elucidating the biochemical and molecular mechanisms of paracrine signaling in endoderm-mesoderm communications [75]. The hepatobiliary-pancreatic axis, consisting of designated endoderm and mesoderm, is designed to mimic the genesis and development of dynamic organs in complex environments, providing opportunities for studying multi-organ diseases in humanoid organoid models [76]. Additionally, it has been demonstrated that inter-organ, cross-lineage communications occur between the co-developing heart and the surrounding endoderm [77]. Although human cardiac organoids show great potential in recapitulating the physiology of the human heart, they often fail to capture the complex crosstalk between the heart and the endodermal organs. Thus, a comprehensive understanding of multi-organ lineages remains elusive. Here, we will expand the application of 3D organoid models in studying interactions between the heart and other endodermal organs.

Heart-foregut axis

A human multilineage pro-epicardium/foregut organoid has been developed to recapitulate the co-emergence of the anterior epicardium, septal mesenchyme, and liver buds. This model investigates its supporting role in epicardium/myocardium development [78]. It primarily involves the co-culture of these multilineage organoids with cardiomyocyte aggregates, forming self-organizing cardiac organoids in which the epicardial-like layer surrounds the myocardial-like tissue. This study offers novel interpretations that advance our understanding of myocardial-foregut interactions during cardiac organogenesis in healthy or diseased environments.

In a related study, Robert Zweigerdt and colleagues encapsulated hiPSC-derived aggregates in Matrigel to simulate early cardiac development, which also forms foregut endodermal tissue, under the influence of Wnt activators and inhibitors [79]. Since the interplay between cardiac mesoderm and foregut endoderm is required for proper myocardial- and endocardial patterning, this model offers a valuable tool for studying in vitro congenital cardiac developmental defects caused by genetic defects. Notably, they observed reduced cardiomyocyte adhesion and hypertrophy in the presence of NKX2.5 mutations, a phenotype similar to the cardiac malformation seen in NKX2.5-knockdown mice. These findings offer new perspectives for understanding developmental and genetic disorders in cardiac biology.

Heart-intestinal axis

Todd C. McDevitt and colleagues developed a multilineage organoid model that recapitulates embryonic heart and gut development [47]. In this model, aggregates consisting of hiPSC-derived mesendoderm progenitors are directed toward a cardiac differentiation fate with ascorbic acid supplementation. The long-term culture of these multilineage organoids successfully mimics the development of embryonic heart and gut. This model suggests that endoderm-derived cells produce paracrine signals and mechanical forces that support cardiac maturation.

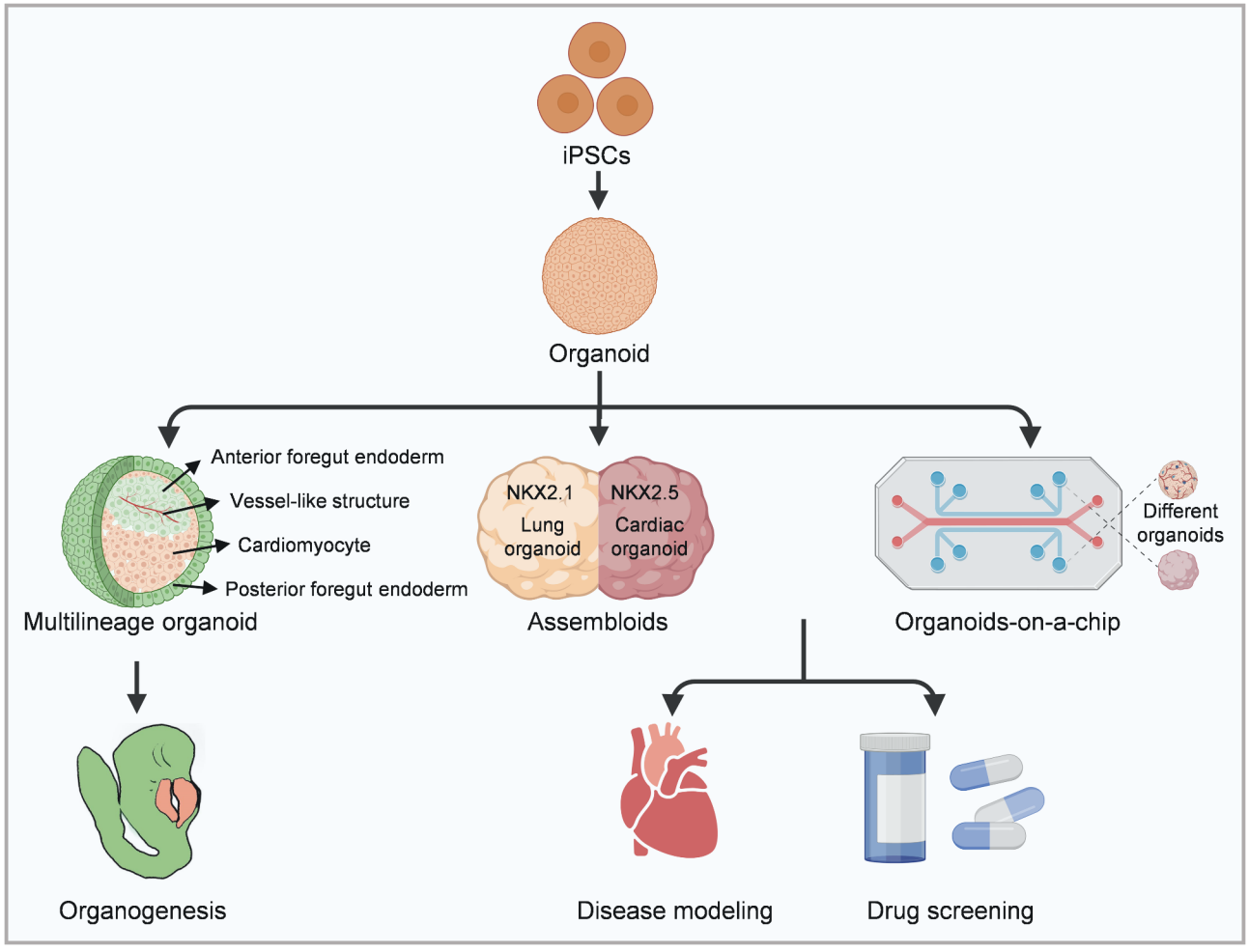

Fig. 1 Humanoid organoid-based models for studying heart-organ interactions. The development of hiPSC-derived multilineage organoid offers a platform for investigating germ layer interactions during cardiac organogenesis. Two primary models including assembloids and organoids-on-a-chip, have been created to model heart-organ interactions under both physiological and pathological conditions, advancing our understanding of underlying mechanisms and supporting drug screening efforts. Created in BioRender. Ni, B. (2024) BioRender.com/p02w371

Table 1 Summary of heart-organ axis interaction models

Organoid models	Strengths	Limitations	Applications
hiPSC-derived multilineage organoids	- Modeling organ development trajectory - Mimicking tissue crosstalk mechanisms - More realistically simulating the internal microenvironment	- Unstable reproducibility - Limited replication of mature structures and functions - Lack of intervention methods due to incomplete understanding of development patterns	- Study of cardiogenesis and germ layer interactions [46, 47, 79] - Studying congenital heart developmental defects [42, 79]
Assembloids	- Modeling direct inter-organ communications - Incorporating more complex cellular components - Exploring gene roles in inter-organ systems	- High heterogeneity - Difficult to standardize culture criteria - Lack of precise quantification techniques	- Studying complex heart diseases [54, 57]. - studying multi-organ system diseases [58, 64]. - Drug screening [57]. - Advancing individualized therapy [54].
Organoids-on-a-chip	- Precisely controlling microenvironment for inter-organ communications - Supporting multi-organ axis studies - Highly reproducible with dynamic monitoring	- Lack of standardized culture protocols - Limited complexity in microenvironment - Challenges with long-term culture and viability	- Modeling multi-organ diseases [93, 106, 107, 175]. - Drug screening [92, 95, 97, 105]. - Advancing personalized precision medicine [95, 101, 107, 175].

Interactions between the heart and intestinal tissues give rise to a more structurally and functionally mature cardiomyocyte population and suggest the induction of an atrial/lymph node differentiation fate.

The multilineage organoid model provides a more direct approach to studying genetic disorders that affect the heart and gut, such as chronic atrial and intestinal rhythm disorders [80]. Furthermore, these multilineage organoids offer a unique opportunity to model tissue morphogenesis or multi-organ complex diseases in vitro, and enable the examination of multi-tissue/organ interactions in the context of embryonic organoid lineage development, physiological maturation, and disease conditions.

Heart-pulmonary axis

As the two major organs in the thoracic cavity, the extensive crosstalk between the developing heart and lungs is crucial for their joint morphological stability and functional maturation. Extensive cardio-pulmonary interactions during organogenesis have been well demonstrated in mouse models [81]. The involvement of pulmonary circulation in cardiac specialization suggests that T-box transcription factor 5, a transcription factor required for cardiac segregation in the second heart field [82], directly drives the cardiac morphogenetic gene regulatory network. Additionally, the conserved retinoic acid-Hedgehog-Wnt signaling cascade coordinates cardiopulmonary development [83]. These pathways initiate a signaling axis of mesoderm-endoderm interactions, providing a molecular basis for the co-evolution of lung and heart structures.

Recent advancements in modeling cardiopulmonary interactions within concentric 3D suspension cultures have further enhanced our understanding [84, 85]. Specifically, Ng WH and colleagues generated heart-lung assembloids by clustering cardiac progenitors and lung progenitors, which were derived from hiPSCs by regulating the Wnt and Nodal signaling pathways, respectively. Their findings demonstrated that the presence of heart tissue accelerated the maturation of lung progenitor cells. Over time, the heart and lung compartments gradually separated, becoming independent. Notably, the activation of exogenous Wnt through GSK-3β inhibition effectively slowed this heart-lung separation, offering valuable insights into the cellular and molecular mechanisms underlying cardiopulmonary co-development and tissue boundary fusion. Although the exact mechanisms remain unclear, this model holds significant clinical potential, particularly in understanding congenital cardiac and pulmonary diseases, such as Diaphragmatic Hernia [86]. Meanwhile, mapping the temporal and spatial relationships between cardiopulmonary and cardiac lineages is expected to provide fundamental insights into the crosstalk essential for organ development.

Heart-organ axis-based cardiovascular disease modeling and drug screening

In addition to studying the intercellular and inter-organ communications during cardiac development, multilineage organoids also offer a platform to simulate the developmental processes of conditions such as innate cardiac dysplasia [87, 88]. Considering the complex microenvironment of the organism, combining existing assembloids with microfluidic technologies enables the establishment of multi-organ disease models. This approach is beneficial for studying mechanisms related to multi-organ system diseases and screening for more effective drugs with fewer side effects, ultimately improving patients' quality of life and survival rates.

Heart-kidney axis

Heart and kidney diseases are closely linked due to their interdependent functions. Cardiorenal syndrome, which involves nephropathy and cardiac disease, represents a common physiological and pathological condition affecting these organs [89]. Dysfunction in the kidneys, through interactions with the circulatory system, usually leads to increased circulating volume and, ultimately, heart failure [90]. However, the complex mechanisms of dysfunction between the cardiac and kidney organoids in multi-organ diseases remain poorly understood [91]. While single-organ models such as cardiac and renal organoids have significantly advanced biomedical research, they cannot capture the dynamic interactions between these organs.

The in vitro models that combine hiPSC-derived heart and kidney organs on a microfluidic chip to form a heart-kidney axis are proving valuable for the study of heart-kidney interactions [92]. These chips typically feature separate channels connecting distinct chambers, with microfluidic channels ensuring the structural and functional integrity of each organ under both static and dynamic conditions. Using this model, the coculture of renal organoids has been shown to reduce the contraction amplitude of cardiac organoids, a phenomenon attributed to nutrient consumption, such as glucose, by the renal organoids [92]. As an in vitro microfluidic platform for the cardiorenal axis, this model enables the investigation of the heart's response to stress or injury and allows for the assessment of how cardiac injury impacts renal tissue, including alterations in renal blood flow, inflammatory responses, and fibrosis [93]. Moreover, from a clinical perspective, this model can be used to investigate the therapeutic effects of cardiac drugs on cardiac tissue and evaluate their possible nephrotoxic effects.

The proximal tubules in the renal unit interact with the vascularization of the cardiac circulatory system through both direct actions (secretion of factors) and indirect actions (glucose and nutrient uptake). However, since cardiac organoids typically lack vascularized structures, combining microarray technology with assembloids is essential to create more complex bionic organoid structures. This integration would better mimic the glomerular and tubule-cardiac circulatory system crosstalk, providing a more accurate representation of the physiological interactions between the heart and kidneys.

Heart-liver axis

Drug-induced hepatic and cardiac side effects are primary challenges leading to drug failures in clinical trials [94]. A multi-organ microarray system developed by Jianhua Qin and colleagues successfully maintained the tissue-specific functions of both cardiac and liver organoids in a co-culture setup [95]. This system was used to assess the liver metabolism in drug-induced cardiotoxicity assays. They found that exposure of Clomipramine, a widely used antidepressant drug, to liver organoids on chip led to impaired cardiac function, significant cardiomyocyte death, and reduced release of calcium fluxes [95]. This is consistent with the side effects observed in clinical patients treated with Chlorpromazine, leading to the conclusion that Chlorpromazine-induced cardiotoxicity is dependent on hepatic metabolism. A multi-organ system of functional, non-invasive recordings has also been devised to study crosstalk between the heart and liver. This model simulates the hydrodynamic impact of drugs in the cardiac and hepatic systems by generating gravity-driven flow through a rocking arm platform, thereby predicting drug metabolism concentration changes more accurately [96].

The liver can metabolize certain drugs, either inactivating or activating their effects. Metabolites produced in the liver, such as Adriamycin, can have toxic effects on the heart, making it essential to characterize liver metabolism when studying the cardiac effects of drugs. Recent articles have summarized recently developed liver and cardiac microarray systems for drug toxicity testing [97]. These systems have shown the potential to predict in vitro action mechanisms and mimic patient-specific differences, suggesting that combining microarray technology with organoid technology is an effective model for in vitro drug screening.

Heart-neuro axis

The heart's innervation is predominantly regulated by the autonomic nervous system, comprising the sympathetic and parasympathetic branches [98]. Changes in neuronal activity significantly affect the structure and function of cardiomyocytes [99]. In turn, heartbeat-induced pressure pulsations have been shown to modulate neuronal activity via mechanosensitive ion channels [100, 101]. These findings illuminate the functional role of coupling between neurons and the heart, highlighting the coordinated responses that may occur during specific activation states. In the neuro-cardiac axis, which mainly involves interactions between the exogenous and endogenous cardiac nervous system, it is essential to simulate changes in the autonomic nervous system at the in vitro level in both normal and pathological states. This approach allows for the study of diseases such as cardiac arrhythmias at a more complex level, focusing on neural regulation rather than direct interventions in the cardiomyocytes [102, 103].

Leveraging the rapid advancements in tissue engineering and organoid technologies, researchers have applied neuro-cardiac organoids on microarrays to demonstrate functional synapses and the contractile coupling properties of cardiac calcium dynamics induced by neuronal stimulation. Specifically, Albano C. Meli and colleagues developed an organ-on-a-chip system in which neuronal cells connected to hiPSC-CMs, forming functional synapses between neurons and cardiomyocytes [99]. From a clinical translational point of view, their models were also tested with acetylcholine analogs: Atropine and Carbachol. They demonstrated that Atropine did not substantially affect the electrical signals of the heart, whereas Carbachol increased the amplitude of calcium transients in cardiomyocytes, potentially suggesting concomitant cardiotoxic effects of Carbachol.

Models are available that incorporate novel engineered materials and developmental features to articulate the neural-cardiac axis [104]. This will further increase the complexity and accuracy of cardiac organoid models in response to autonomic nervous system innervation. Ultimately, this integration contributes to the development of more effective therapeutic strategies, which can reveal the specific effects of the autonomic nervous system on cardiac function.

Multi-organ interactions

Organs communicate with each other primarily through specific tissue boundaries, unique inter-organ barrier effects, and crosstalk facilitated by vascular flow. Diseases affecting multiple organ systems pose significant challenges in simulation, and there remains a lack of in vitro models that can accurately represent the interactions of human tissues across various organs, particularly humanoid disease models.

A three-tissue organ-on-a-chip system, comprising liver, heart, and lung organoids, has been developed to study inter-organ responses to drugs and other compounds [105]. In this system, bioengineered tissue organoids and tissue constructs are integrated within a closed

circulatory perfusion system, replicating the interactive nature of the human body. An unanticipated side effect of Bleomycin, cessation of cardiac organoid beating, was detected in the three-tissue organ-on-a-chip system. This side effect was not merely due to Bleomycin but potentially caused by a secondary factor produced by one of the other tissues in the platform, since this effect was not observed in a cardiac-only system, highlighting the value of multi-organ interaction models [105].

Recently, Aleksander Skardal's lab expanded the integrated system to incorporate six humanized constructs, including liver, cardiac, lung, endothelium, brain, and testes organoids [106]. Drug toxicity assessment in the six-organoid system demonstrated that the anticancer drug Ifosfamide exhibits significant neurotoxicity when liver organoids are present. These findings underscore the importance of liver-associated metabolism in the neurotoxic effects of circulating Ifosfamide.

Given that blood flow is intricately linked to the development of CVDs, a multi-organ model featuring the vascular barrier was also created. This model incorporates mature myocardial, liver, bone, and skin tissues within a multi-organ microarray to detect inter-tissue crosstalk effects. This system, using fluorescent markers secreted by different tissues, unexpectedly revealed the involvement of immune cells in the vascular barrier [107, 108]. Further studies using inter-organ tissue microarrays hold promise for more accurately modeling multi-systemic diseases such as CVDs, for identifying early biomarkers for drug toxicity [109].

In summary, multi-organ microarray systems, particularly those incorporating the heart, are essential for evaluating the safety and efficacy of drugs that have direct or indirect effects on cardiac function. As AI-driven big data technologies and advanced histology techniques continue to evolve, these systems are poised to enhance the success rate of new drug development.

The maturation of cardiomyocytes in hiPSC-derived cardiac models

Although hiPSC-derived cardiac organoids have emerged as a promising tool for studying cardiac physiology and pathology, and inter-organ communications, cardiomyocytes in these organoids exhibit several immature and fetal-like features that limit their functional resemblance to adult cardiomyocytes. Understanding these differences is crucial for advancing their application in disease modeling and therapeutic interventions.

Immature features of hiPSC-derived cardiomyocytes

Morphology Mature human cardiomyocytes typically exhibit a unique and well-defined rod-like structure, with a diameter of 15–30 μm and a length of 100–150 μm, which is essential for normal cardiac function [110]. In contrast, hiPSC-CMs are smaller, with diameters ranging from 5 to 10 μm, and often display irregular, round shapes [111]. Meanwhile, hiPSC-CMs exhibit irregular sarcomeres and T-tubules, as well as less abundant mitochondria and gap junctions, leading to a disorganized contraction. Altogether, these morphological differences influence their electrophysiological and contractile properties, thereby limiting their utility in disease modeling.

Electrophysiology The electrophysiological properties of cardiomyocytes are vital for normal heart function and in response to pathological states. hiPSC-CMs often exhibit an imbalance between sodium-calcium exchanger and L-type Ca^{2+} channel-mediated inward currents and potassium ion efflux, which affects cardiac electrophysiologic activity and contractile function [112]. Unlike the adult ventricular cardiomyocytes, which are electrically quiescent until triggered by a neighboring cell, the immature hiPSC-CMs exhibit also autorhythmicity. This characteristic is due to the high levels of hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4) in the plasma membrane and the spontaneous leak of calcium from the sarcoplasmic reticulum [113].

Metabolism During maturation, cardiomyocytes transition from glycolysis to fatty acid β-oxidation as their primary energy source, accompanied by increased mitochondrial density and more developed cristae structures [114]. In contrast, immature cardiomyocytes predominantly rely on glycolysis, with weaker mitochondrial oxidative phosphorylation and lower ATP production, for energy supply [115]. This metabolic immaturity may lead to biases in drug toxicity assessments and hinder effective clinical translation.

Excitation-contraction coupling property Excitation-contraction coupling properties are fundamental to cardiomyocytes and are essential for other electrophysiological and mechanical cellular properties. Mature cardiomyocytes utilize T-tubular junctions for synchronized calcium release during action potential conduction, maintain high sarcoplasmic reticulum calcium content, and exhibit tight coupling of L-type calcium channels to ryanodine receptors to sustain Ca^{2+} kinetic equilibrium [116]. hiPSC-CMs, however, possess immature T-tubular structures, affecting intracellular calcium release and resulting in immature calcium transients and conditional dependence on calcium cycling. These immaturity challenges the accurate replication of mechanical characteristics observed in the adult heart.

Methods and challenges in maturing hiPSC-derived cardiomyocytes

To enhance the maturity of hiPSC-CMs, a variety of strategies have been proposed to better mimic the physiological environment or modulate key signaling pathways. These methods primarily focus on long-term culture, biophysical stimulation, cell co-culture, and 3D culture. While promising, each of these strategies presents its own challenges and limitations that must be addressed to optimize their application in disease modeling, drug screening and regenerative medicine.

Long-term culture Long-term culture is a well-established approach to promote the maturation of hiPSC-CMs by closely mimicking the time course of cardiac development. After more than 60 days of culture, hiPSC-CMs show improved myofilament organization, and undergo a metabolic shift, with increased mitochondrial number, mature cristae structures, and more efficient ATP production. A significant improvement in contractility and calcium-handling capacity will be also observed in hiPSC-CMs after a long-term culture [113, 117, 118].

Biophysical stimulation Biophysical stimulation, including mechanical and electrical signals, is another promising strategy to drive the maturation of hiPSC-CMs by mimicking the dynamic mechanical and electrophysiological microenvironment of adult cardiomyocytes. Adult cardiomyocytes are subjected to cyclic stretch and fluid shear forces during cardiac systole and diastole, which are essential for regulating gene expression and functional maturation [119]. By applying cyclic stretch to hiPSC-CMs, their myotome length increases, and the alignment of transverse striations is enhanced [120]. Meanwhile, electrical stimulation promotes the maturation of action potentials and improves calcium transient synchronization in hiPSC-CMs [121].

Co-culture and 3D culture Co-culture and 3D culture are advanced strategies designed to more accurately replicate the cardiac microenvironment by fostering physical, chemical, and intercellular interactions that promote maturation. Co-culture with other cell types, such as cardiac fibroblasts, endothelial cells, and mesenchymal stem cells, provides paracrine signaling and direct cell-to-cell interactions that support maturation [122–124]. For instance, co-culturing hiPSC-CMs with cardiac fibroblasts promotes functional coupling, reduces spontaneous beating frequency, and prolongs action potential duration [122]. 3D culture systems, through mimicking the cell component and mechanical properties of myocardial tissues, could promote the development of formation of T-tubule systems and accelerate the maturation process. Recent advancements in 3D co-culture systems show great promise in enhancing maturation and reducing the time required for functional development [122, 125, 126].

Despite significant progress, existing methods to promote the maturation of hiPSC-CMs still face limitations, particularly in replicating adult myocardial tissue's contractility, metabolic capacity, and functional maturation. Long-term culture is time-consuming and complex, with the potential for cell aging or functional degradation [127]. While co-culture and 3D culture systems offer more in vivo-like representations, challenges persist in optimizing cell ratios (e.g., avoiding fibroblast-induced fibrosis) and managing the heterogeneity that may interfere with drug screening or signaling studies [128]. 3D culture further faces issues such as technical complexity in vascularization and nutrient/oxygen gradients leading to cell necrosis [57, 129]. Additionally, the lack of standardized stimulation parameters and the need for scalable bioreactors complicate the process, with overstimulation potentially causing cellular damage and hindering effective maturation [123]. Variations in cell lines or culture conditions can also lead to inconsistent maturation, affecting disease modeling accuracy.

Application limitations of immature cardiomyocytes

Given the ongoing challenges in achieving adult-like hiPSC-CMs, their limitations must be carefully considered when applying these hiPSC-derived cardiac models, as outlined in the following sections.

Inadequate functional simulation

Immature hiPSC-CMs exhibit weak and uncoordinated contractility, which limits their ability to replicate the synchronized contractile behavior of adult myocardium. This immaturity reduces the sensitivity of models in assessing myocardial dysfunction, particularly in diseases like heart failure [130]. Additionally, the electrophysiological properties of immature hiPSC-CMs differ significantly from those of adult cardiomyocytes, with notable discrepancies in action potential morphology and ion channel expression [131]. These differences impair the ability of hiPSC-CMs to coordinate electrophysiological activity with cells from other organ systems, such as neurons, thereby affecting the accuracy of inter-organ signaling simulations [132].

Bias in disease modeling

In models of inherited cardiomyopathies, such as hypertrophic cardiomyopathy (HCM), the immaturity of hiPSC-CMs may obscure the phenotypic expression of disease-causing mutations. For instance, mutations in sarcomeric proteins that lead to severe dysfunction in mature tissues may induce minor contractile abnormalities in immature cells [133]. Furthermore, variations in cell lines or culture conditions can lead to differences

in maturation, exacerbating biases in disease modeling [127]. The paracrine signaling profile of hiPSC-CMs also diverges from that of adult cardiomyocytes, which can distort disease-related signaling pathways. For example, cytokines like IL-6, secreted by hiPSC-CMs, may induce aberrant inflammatory responses in co-cultured cells, confounding disease modeling and therapeutic testing [134].

Biased prediction of drug responses

The metabolic and electrophysiological immaturity of hiPSC-CMs can result in inaccurate predictions of drug toxicity. For example, the cardiotoxicity of certain chemotherapeutic agents is linked to mitochondrial function, but the glycolysis-dependent metabolism of immature hiPSC-CMs may lead to false-negative results [124]. In models of diseases like HCM, abnormal contraction patterns may not fully reflect the clinical condition of patients, thereby impairing the accuracy of drug testing [135]. Furthermore, metabolic differences between hiPSC-CMs and adult cardiomyocytes can limit the relevance of these models in studying the heart's interaction with other metabolic organs (e.g., liver), which may obscure the effects of drug metabolism on cardiac function [136].

In summary, the immature characteristics of hiPSC-CMs impact the performance, reliability, and predictive accuracy of hiPSC-derived cardiac models in modeling cardiac physiology and pathology. To improve the maturity and application value of these models, multifactorial optimizations are necessary. An integrated approach, combining biomaterials, mechanical stimulation, metabolic modulation, and AI-assisted optimization, may help overcome these challenges and facilitate the effective application of hiPSC-CMs in precision medicine and translational research.

Application opportunities of immature cardiomyocytes

Despite their structural and functional immaturity, the unique features of hiPSC-CMs may not be a critical limitation in some applications and can even offer distinct advantages in certain application contexts. The following sections discuss how these immature characteristics can be effectively leveraged for specific applications.

Developmental biology and abnormality studies

The molecular and metabolic characteristics of hiPSC-CMs closely resemble those of fetal cardiomyocytes, making them ideal for constructing models of early cardiac development and associated developmental abnormalities. For instance, hiPSC-CMs have been utilized to investigate the pathogenesis of cardiac septal defects caused by embryonic GATA4 mutations [137]. Additionally, cardiac organoid models combined with machine learning have been used to study ventricular developmental defects caused by NKX2-5 deficiency [138]. Researchers have also successfully modeled cardiac outflow tract anomalies associated with DiGeorge syndrome, shedding light on the role of T-box transcription factor 1 in developing the cardiac conotruncal arterial trunks [139].

Modeling specific pathological features

Immature hiPSC-CMs are particularly valuable for modeling the early pathological features of specific cardiac diseases, such as inherited arrhythmias and cardiomyopathies, which are more easily triggered and observed in these cells [140]. For example, exposing hiPSC-CMs harboring a ryanodine receptor 2 (RYR2) mutation to β-adrenergic stimulation recapitulated the calcium spark abnormalities and delayed afterdepolarizations observed in patients with catecholaminergic polymorphic ventricular tachycardia, highlighting their propensity for arrhythmias [141].

Modeling non-myocyte-associated cardiac diseases

Non-myocyte cell populations in the heart, including fibroblasts, vascular cells, autonomic neurons, and immune cells, also play crucial roles in cardiac homeostasis and disease. For example, microvascular complications and neurocardiac dysfunction are commonly reported in patients with Type 2 Diabetes Mellitus, suggesting the involvement of non-myocyte cells in the disease process [142]. Cardiac organoids, which typically incorporate hiPSC-CMs along with additional cell types, offer a promising platform for studying the pathological mechanisms associated with these non-myocyte cells. Meanwhile, certain drugs may exert their effects on non-myocyte cell types. Recent studies have shed light on the potential mechanism of SLGT2 (sodium-glucose co-transporter-2) inhibitor-mediated cardioprotection, which may involve direct or indirect effects on the cardiac non-myocyte populations or even extra-cardiac tissues [142]. Therefore, despite the immaturity of hiPSC-CMs, hiPSC-derived cardiac organoids remain a valuable model for investigating the non-myocyte-related cardiac response to drugs or signals from other organs.

Drug screening and toxicity testing

Immature cardiomyocytes are more sensitive to drug-induced effects, making them valuable tools for detecting early toxicity or efficacy signals that may be missed in mature cardiomyocytes. For instance, screening for cardiotoxicity of the antiviral drug Raltegravir using hiPSC-CMs revealed a prolongation of the action potential duration, suggesting a potential arrhythmogenic risk [143]. These cells are also suitable for modeling hypersensitivity in pediatric hearts to drugs, such as the early

cardiotoxicity of the chemotherapeutic agent Adriamycin [144].

In summary, rather than being a limitation, the immaturity of hiPSC-CMs offers unique advantages in modeling heart development during early stages and constructing sensitive disease models. By precisely regulating their maturation, the application potential of hiPSC-CMs in personalized medicine and translational research can be further expanded.

Omics tools for the integrative study of inter-organ communications

Studying inter-organ communications is essential for understanding complex physiological and pathological processes. Thus, comprehensive high-throughput techniques are indispensable for the integrative analysis of these interactions. Single-cell and spatial transcriptomics are current cutting-edge technologies offering detailed descriptions of physiological and chemical responses within tissues [145, 146]. These techniques enable the identification of biomechanical cues that facilitate cross-lineage interactions and predict signaling networks for tissue interactions [147]. By applying these techniques to the hiPSC-derived inter-organ interaction models, researchers can better understand the complex processes regulating multi-organ interactions during embryogenesis and disease.

Single-cell transcriptomics technology

The most established single-cell histological approach, single-cell transcriptomics technology, has recently begun to examine organogenesis with unprecedented resolution. Specifically, it projects the transcriptional profile of the paracrine signaling pathway into the progenitor gene lineage, inferring a roadmap of endoderm and mesoderm-induced interactions that orchestrate organogenesis [148]. Additionally, single-cell transcriptomics can model intra-organ crosstalk and chemotaxis-directed effects in the cardiac lineage, detecting patterning processes during human cardiac development [87, 149]. The full-cycle multi-omics map of cardiac development has been constructed by multi-omics technology [150]. A high-resolution spatial single-cell map of the developing human heart has been created by integrating single-cell sequencing with high-resolution in situ hybridization [151]. This map serves as a platform for drug screening and multiorgan systemic disease treatment by pooling expression communities across different organs to construct a comprehensive multi-organ profile of the cardiac-organ axis.

Single-cell transcriptomics allows for highly resolved, revealing the development and fate of lineages in organogenesis. Current genealogical recorders that combine reporter barcodes [152] with inducible CRISPR/Cas9 [153] can dynamically track clones within the initial hiPSCs pool. These methods map the dynamic states of multilineage organoids through detailed cellular and molecular roadmaps of cell fates [154]. Moreover, simulations of cellular movements and germ layer interactions in the formation of zebrafish protocorm embryos, achieved through single-cell transcriptome and in situ hybridization analyses, provide insights into head formation [155]. These approaches offer profound insights into lineage specification coordination and associated paracrine signaling, enhancing our understanding of the complex processes regulating inter-organ communications during embryogenesis.

Spatial transcriptomics technology

Cardiovascular diseases are the leading cause of death worldwide, with high morbidity and mortality rates. Emerging spatial transcriptomics techniques enable the elucidation of cellular spatial localization and the construction of dynamic 3D single-cell transcriptional profiles. This approach reveals the cellular spatial location and structure in the continuous progression from angina pectoris to myocardial infarction to heart failure, ultimately identifying new therapeutic targets [154]. In addition, the current spatiotemporal transcriptional profiles involve precise interactions between cell types in the three germ layers of gut development, which map all gut compartments and highlight coordinated events and crosstalk effects [156]. Notably, single-cell and spatial transcriptomic analysis allows for a comprehensive classification of neurons and neuronal circuits in the brain, enabling the reconstruction of multiple temporal and spatial dimensions. By integrating metabolomic profiles with genetic and epigenetic modifications, this approach offers new insights into the mechanisms underlying neurological disorders [157].

Spatial transcriptomics technology demonstrates the spatiotemporal mapping of human embryonic development and various multi-organ diseases, providing precise localization of gene expression and intercellular interactions. The capability is beneficial for presenting the key regulatory pathways of specific molecular interactions in multi-organ diseases [158]. Spatial transcriptomics provides new insights into disease genesis and elucidates changes in spatiotemporal effects among different organs at the multi-organ level.

Metabolomics technology

Organoids offer an ideal platform for drug screening, and exploring metabolomic regulation or metabolite transport mechanisms under both normal and pathological conditions. In particular, patient-derived organoids are invaluable for tailored personalized treatments, as they

enable the identification of specific or optimal drugs for individual diseases or patients [159].

Mass spectrometry (MS) remains the cornerstone of metabolomics due to its unparalleled sensitivity, selectivity and capability for identifying metabolites. Among the available methods, liquid chromatography-mass spectrometry (LC-MS) is the most widely used for studying metabolomic regulatory mechanisms in complex biological systems. LC-MS facilitates the analysis of intricate mixtures such as organoids, allowing researchers to map metabolic pathways, discover biomarkers, and better understand disease progression [160]. Additionally, gas chromatography-mass spectrometry complements LC-MS by enabling targeted analysis of specific drugs and their metabolites.

Recent advancements have extended metabolomics to investigate interactions between the gut and brain axis interactions through complex organoid models [161]. In microbe-host interaction studies, changes in the microbial populations with related to metabolites such as brain neurotransmitters can be quantified using high-throughput LC-MS techniques, providing valuable insights into neurological diseases. Furthermore, the influence of the gut microbiome on cardiovascular health is increasingly evident. Short-chain fatty acids produced by gut microbes are known to regulate blood pressure through various receptors and pathways [162]. Studying the pathological responses of cardiac organoids to short-chain fatty acid stimulation offers a promising avenue for understanding the gut-heart axis and the mechanisms underlying CVDs.

Metabolomics in organoids also represents exciting possibilities for integrative systems biology. By integrating metabolomics with multi-omics technologies, including whole-genome sequencing, transcriptomics, ATAC-seq, and untargeted metabolomics, a multi-dimensional data platform can be developed. This platform enables a more comprehensive understanding of inter-organ communications in the human body and advances the potential of personalized medicine applications [163].

Proteomics technology

Proteomics analysis offers a powerful tool for uncovering intercellular signaling pathways and functional protein networks in health and disease. When applied to 3D organoid models derived from human cells, proteomics provides profound insights into the molecular mechanisms underlying clinical conditions [164].

Several primary approaches are currently used in proteomics. The most widely employed method is high-throughput analysis via MS, which relies on liquid chromatography-chromatography and signal capture based on fragmented peptide sequences and spectral counting [165]. This technique can identify thousands of proteins, allowing researchers to target key pathways effectively. Antigen-antibody array technology is suitable for medium- to low-weight proteomics analysis. It captures signals from fluorescence, chemiluminescence, or oligonucleotide-coupled tag labeling [166]. This method enables the study of protein-protein, lipid, small molecule, nucleic acid and antibody interactions. Finally, reversed-phase protein array technology facilitates large-scale protein sample analysis. It captures quantitative signals through colorimetric amplification or fluorescence detection, making it suitable for tracking proteins in signaling networks and studying post-translational modifications, including phosphorylation, methylation, and acetylation [167].

Organoid systems incorporating multiple cell populations further enrich proteomics research by closely mimicking the complexity of human tissues. Multidimensional proteomics analysis, combined with innovative predictive algorithms, will provide a more accurate representation of organism complexity in disease environments, holding great promise for advancing clinical drug development and therapy [168]. As organoid technologies evolve, the scalability and reproducibility of proteomics applications are expected to be improved. These advancements will provide unprecedented opportunities for drug development, biomarker discovery and the design of targeted therapies, ultimately translating into better clinical outcomes.

Conclusion and perspectives

This review illustrates the application of hiPSC-derived models for studying interactions between the heart and other organs at both 2D and 3D levels. We also introduce advanced tools for analyzing these interactions, focusing on the coordinated interactions in organ development. Critical methodologies include single-cell and spatial transcriptomics, which identify potential action targets and assess the reciprocal effects between the heart and other organs (Fig. 2).

When employing 2D cell cultures or 3D organoid models, extensive measures are necessary to improve cell maturation and overall tissue complexity to mimic human physiology and pathology accurately. Researchers are continuously exploring novel organoid models and innovative practices that combine organoids with the immune system, as summarized in a recent review [169]. With the advancement of microfluidic devices, real-time imaging, and 3D printing, combining organoids with co-culture strategy will further deepen researchers' understanding of organ development, disease onset, and progression. Newly developed organoid chip technologies show great potential for constructing higher fidelity organ models, and modern Organoids-on-a-chip systems

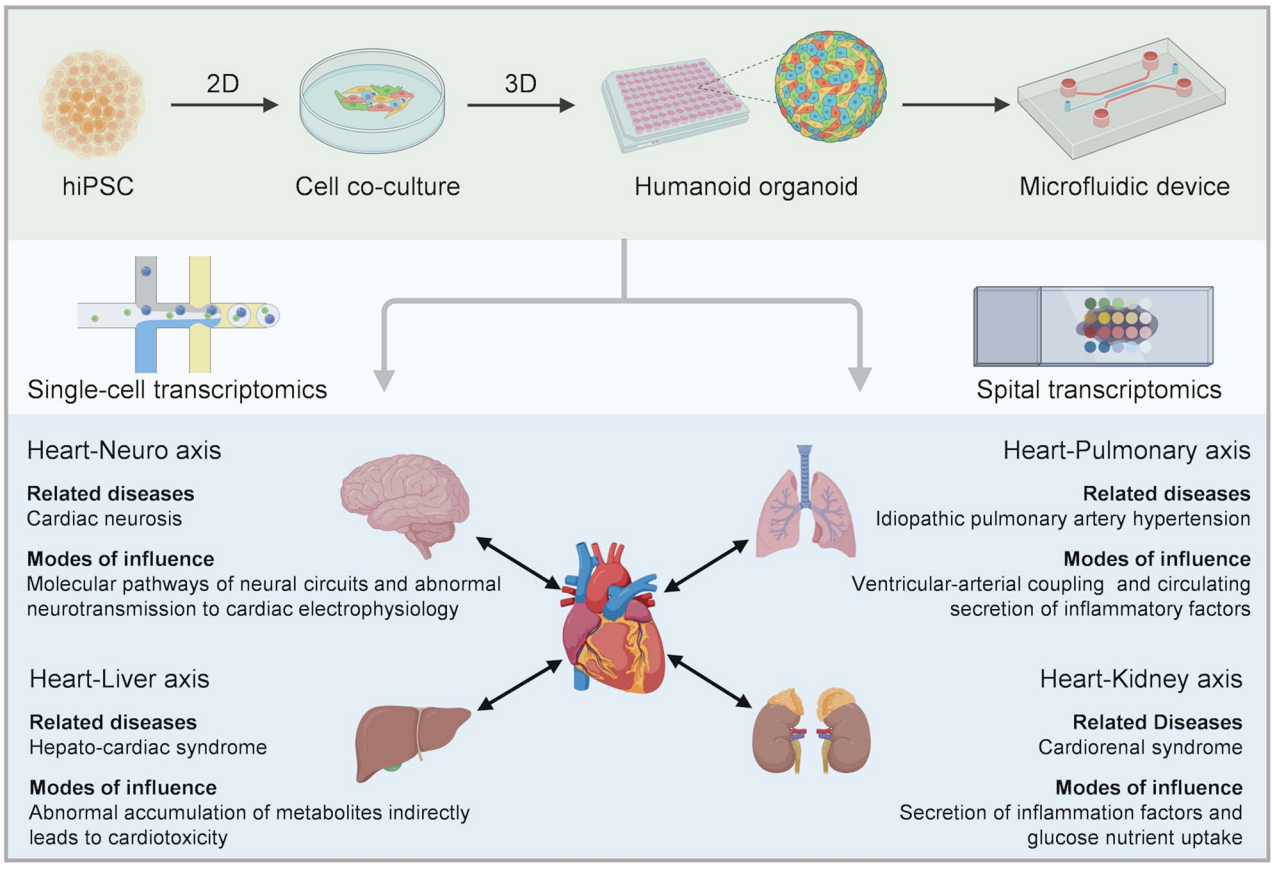

Fig. 2 Advances in heart-organ interaction studies using hiPSC-derived 2D and 3D models. With advancements in hiPSC-derived 2D cell co-culture systems, 3D humanoid organoids and microfluidic chip technologies, alongside emerging high-throughput sequencing technologies, it is now possible to investigate heart-organ communications in heart-involved multiple organ dysfunction syndromes in vitro. Created in BioRender. Ni, B. (2024) BioRender.com/a92e049

hold great promise for elucidating the mechanisms of systemic diseases [170, 171].

Despite their potential, organ microarrays are still relatively new in CVD research, with limited studies on cardiac organoids. Many CVDs require modeling their complexity at the multi-organ level, especially heart-brain level interactions, which affect inter-organ crosstalk in an interrelated manner through neurotransmission [172]. Transplantation of cardiac organoids has been carried out, and the highlight of cardiac organoid transplantation is the effect of enhancing the maturation of cardiomyocytes to achieve a molecular, structural, and physiological phenotype similar to that of the adult heart [173]. Notably, researchers have demonstrated that cardiac organoid transplants can restore the contractile function of injured primate hearts [174]. Future challenges may include the integration of biosensors and the development of complex constructs for cardiac-engineered tissues. We anticipate that the ongoing development of hiPSC-based organoid models and advanced technologies will further enhance our understanding of CVDs.

Abbreviations

2D	Two-dimensional
3D	Three-dimensional
AI	Artificial intelligence
CCIs	Cell-cell interactions
CX43	Connexin 43
ECM	Extracellular matrix
ICAM-1	Intercellular cell adhesion molecule-1
LC-MS	Liquid chromatography-mass spectrometry
IL-1β	Interleukin-1beta
iPSC-CMs	Induced pluripotent stem cell-derived cardiomyocytes
MS	Mass spectrometry
ROS	Reactive oxygen species
SCN1A	Sodium voltage-gated channel alpha subunit 1
αSMA	Alpha-smooth muscle actin
TNF-α	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor

Acknowledgements
The authors declare that they have not use AI-generated work in this manuscript.

Author contributions
B.N. and L.Y. reviewed the literature and drafted the manuscript; B.N. and Y. Z. designed the figures; S.H. and W.L. provided supervision and revised the manuscript. All authors approved the final manuscript.

Funding
This work was supported by National Key R&D Program of China (2022YFA1104300, 2021YFA1101902), National Natural Science Foundation of China (82241202, 82170364, 81970223, 82200339), Natural Science Foundation of Jiangsu Province (BK20240001), the Space Medical Experiment Project of China Manned Space Program (HYZHXM01018), Jiangsu Cardiovascular Medicine Innovation Center (CXZX202210), National Center for International Research (2017B01012), and A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

Data availability
Data sharing is not applicable to this article as no new data were created or analysed in this study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 4 November 2024 / Accepted: 13 March 2025
Published online: 28 March 2025

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