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PERSPECTIVE

Technologies and applications of current human cardiac organoids



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Abstract Human self-organizing cardioids, a recent breakthrough in cardiac organoid research, are constructed with the specialized cardiac lineage cells derived from human pluripotent stem cells (hPSCs) and have made rapid advancements since 2021. A key advantage of these organoids is their minimal reliance on external interventions, allowing them to more accurately replicate the heart's developmental processes through intrinsic signaling pathways, thereby closely mimicking natural cardiac characteristics. Consequently, they hold significant promise for improving drug safety evaluations, treating both congenital and acquired heart diseases, advancing eugenics practices, developing humanized cardiac disease models, conducting research in regenerative medicine, and understanding how unique environments (such as aerospace) affect human health. This review systematically describes the current various self-organizing cardioid construction techniques, comparing the structural differences caused by diverse signal stimulations, which would be instrumental in optimizing designs for more advanced and mature cardioids. Additionally, we summarize existing applications and address the challenges faced. Despite some uncertainties and challenges in current technologies and applications, this emerging cardiac organoid technology holds promise to provide new possibilities for cardiovascular medicine through continuous refinement.

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1. Introduction

As the first functional organ to develop during embryonic development, research on the heart in a three-dimensional (3D) cultured field can be traced back to 1993, when Maltsev et al.¹ utilized pluripotent embryonic stem cells (ESCs) to differentiate into embryoid aggregates containing spontaneously beating cardiomyocytes. The term “organoids” was proposed in 2009, and subsequent reports have described various organoids, including intestine, stomach, retina, brain, lung, liver, pancreas, and others^{2–6}. However, human cardiac organoids (hCOs, also referred to as cardioids) were not gradually established until 2017. Currently available hCOs sources can be broadly categorized into four groups: (1) cardiac microtissues formed by proportionally mixing different types of cardiac cells^{7,8}; (2) engineered heart tissue⁹, and microfluidic heart on-chip¹⁰; (3) 3D bioprinting heart^{11–13}; (4) self-organizing cardioids relying on the inherent ability of stem cells to differentiate in an organized manner^{14,15}. Like human brain organoids applications demonstrated by our group^{16–21}, the hCOs provide versatile platforms for modeling and investigating congenital/acquired heart diseases, drug screening/testing, tissue regeneration and transplantation, additionally, holding potential in other fields such as eugenics and aerospace medicine research. Therefore, it is imperative to establish and enhance this technology.

Human self-organizing cardioids, a recent breakthrough in hCOs, spontaneously aggregate from ESCs or induced pluripotent stem cells (iPSCs) upon stimulation by various signals. These cardioids exhibit automatic beating, induce differentiation, and some even form chamber-like structures. Since the pioneering research by Hofbauer et al.¹⁴ in 2021, numerous experimental methods have emerged that highlight distinct cardiac characteristics. Compared to other categories, self-organizing cardioids possess the advantage of simulating embryonic development and closely resembling native hearts through minimizing artificial intervention, aiming for comprehensive replication of cardiac characteristics as much as possible. Consequently, the self-organizing mode has become a crucial technology.

In this paper, we first examine the current classification of human self-organizing cardioid models based on their structural characteristics, highlighting the distinct construction features among various technologies and comparing these differences with embryonic development processes. This is of crucial importance for researchers to optimize technologies with more targeted structures and characteristics on the existing basis. Subsequently, we review the application status of existing self-organizing cardioid models in areas such as disease modeling, pathogenesis research, drug safety and toxicity assessment, as well as cardiac regeneration and transplantation. We also provide a comprehensive evaluation of the future development directions and application prospects of the cardioids.

2. Current self-organizing cardioid construction methods

Since 2021, human self-organizing cardiac organoid models, incorporating multiple regulatory factors of embryonic heart development signals, have been successively published (Tables 1 and 2^{14,15,22–39}). Although there still exists a substantial disparity compared to the native heart, multiple advantages have been demonstrated.

2.1. Basic scheme of constructing human self-organizing cardioids

The embryonic heart develops from the mesoderm^{40,41}. WNT signaling, transmitted from the adjacent endoderm, plays a crucial role in regulating cardiomyocyte differentiation (Fig. 1)^{42,43}. Based on this principle, Drakhlis et al.²² attempted to promote the development of heart-forming organoids from human pluripotent stem cell (hPSC) aggregates embedded in Matrigel through a biphasic WNT (activation/inhibition) signal. This approach resulted in heart organoids with self-assembling and layered, consisting of an inner core (endodermal foregut-like cells and endothelial cells), a dense layer of cardiomyocytes, and an outer layer (septum-transversum-like cells, cardiomyocytes, liver anlagen, and mesenchymal cells) (Fig. 1)²². These structures closely resemble the early native heart and embryonic foregut, effectively recapitulating early heart and foregut development⁴⁴. However, it has yet to develop distinct cardiac chambers. Another method developed by Hofbauer et al.¹⁴ addresses this problem. They introduced additional stimuli from cardiac embryonic development signal regulators (Table 1), promoting the differentiation of three primary cardiac cell types—cardiomyocytes, endothelial cells (ECs), and fibroblast-like cells—and the formation of spontaneously beating chambers¹⁴. This process requires the WNT-BMP signaling axis and the downstream transcription factor *HAND1* for cavity morphogenesis (Fig. 1)¹⁴. Almost simultaneously, Lewis-Israeli et al.²³ reported a high-throughput human heart organoid method using hPSCs to mimic fetal heart properties through self-assembly and developmental signals (WNT activation/inhibition/activation). The organoid recapitulates cardiac field formation and atrioventricular specification, develops a complex vasculature, and exhibits robust electrophysiological activity with sarcomere formation and strong, regular beating around the large central compartmental structure²³. These are performed by well-organized multilineage cardiac cell types, comprising approximately 59% cardiomyocytes, 16% epicardial cells, 14% endocardial cells, 12% cardiac fibroblasts, and 1.6% ECs, which contain the major cells in the heart^{23,45}. Notably, the cardioid showed a high vascular branching point without additional steps²³, warranting further investigation into its functional implications and the developmental origins of cardiac vascularization. The self-organizing cardioid construction methods by Hofbauer et al.¹⁴ and Lewis-Israeli et al.²³ have paved the way for improving and enhancing artificial hCOs. Volmert et al.²⁴ further optimized the design of Lewis-Israeli et al.²³ by implementing an enhanced maturation medium to mimic in utero conditions after initially establishing a human heart organoid. They successfully generated gestational hearts that closely resembled those of 6–10-week-old fetuses, including progressive mitochondrial, metabolic, and electrophysiological maturation, as well as anterior-posterior heart tube patterning²⁴. It might serve as a robust tool for the research of fetal heart disease.

Human embryonic organoid technology has successfully replicated the development of the three germ layers (ectoderm, mesoderm, and endoderm)⁴⁶. And all methods described above were developed using hPSCs-derived mesoderm (Fig. 1). Does this mean a cardioid from mesoderm is inherently superior? Song et al.²⁵ compared self-organizing capabilities between cardiac mesoderm cell-derived COs and cardiomyocyte-derived COs.

Table 1 Literature summary of self-organizing cardiac organoids.

Year & Authors	Model	Sp. Cell types & Scaffold	Signal stimulus	Characteristics (Morphological structure & cellular composition)	Cardiac chamber & Spontaneous beating	Electrophysiological properties	Application	Ref.
2021 Drakhlis et al.	Heart-forming organoids (HFOs)	H hPSCs aggregates; Matrigel	Biphasic WNT (activation/inhibition) signal; CHIR, IWP2	Highly similar to early embryonic heart and foregut anlagen, including myocardial layer lined with endocardial-like cells, anterior foregut endoderm, vessel-like structures, and cardiomyocytes, all surrounded by septum transversum-like cells, cardiomyocytes, and posterior foregut endoderm (liver anlagen) and covered by mesenchymal cells.	✕ ✓	Mainly exhibit immature ventricular-like low upstroke velocity electrophysiological characteristics.	Cardiogenesis; Non-compact HCM	22
2021 Hofbauer et al.	Chamber-like cardioids	H hPSCs aggregates; ✕	WNT-BMP signal axis; Cardioids: CHIR, FGF2, LY294002, Activin A, BMP4, Ins, VEGF-A, IWP2; Epicardium: CHIR, FGF2, LY294002, Activin A, BMP4, Ins, RA, IWP1; Co-culture: SB431542, insulin.	Early ventricular heart chamber comprised three cardiac lineages, cardiomyocytes, ECs, and fibroblast-like cells; Compatible epicardium aggregates were added, spontaneously spreading to cover the cardioid, interacting with the cardiomyocyte layer, migrating inward, and differentiating.	✓ ✓	Early left ventricular-like action potential waveforms and electrical activities	Cardiogenesis; Cryo-injury and CHD	14
2021 Lewis-Israeli et al.	Human heart organoids (hHOs)	H hPSCs aggregates; ✕	Three-step WNT signaling axis (activate/inhibit/activate), BMP signal axis; CHIR, BMP4, Activin A, Wnt-C59	Mimic fetal heart properties through self-assembly, activating developmental signals. Recapitulates cardiac field formation and atrioventricular specification using multiple cardiac cell types, including cardiomyocytes, epicardial cells, endocardial cells,	✓ ✓	Normal electrophysiological activity with well-defined action potential waves (QRS complexes, T and P waves) and regular action potentials, and strong regular calcium waves typical of cardiac muscle.	Cardiogenesis; Pregestational diabetes-induced cardiomyopathy	23

2023 Volmert et al.	hHOs	H	hPSCs aggregates; ✗	At the early stage of differentiation: Follow the protocol of Lewis-Israeli et al. ²³ ; Days from 20 to 26: Palmitate-BSA, oleate-BSA, lineolate-BSA, L-Carnitine, T3 hormone, ascorbic acid, glucose, LONG R ³ IGF1; Days from 26 to 30: Same as before, except without LONG R ³ IGF1.	fibroblasts, and ECs; Exhibits robust electrophysiological activity with well-defined fibro-sarcomere and developed vasculature. Optimized based on Lewis-Israeli et al., multiple chemical stimuli were added between days 20–30 to promote hHOs maturation; hHOs closely resembling 6–10-week-old gestational hearts, with large atrial, ventricular chambers and beating, and mimic the post-heart tube stage; Contained various cell types similar to early embryonic hearts, including vCMs, aCMs, valve cells, proepicardial-derived cells, conductance cells, epicardial cells, proepicardial cells, etc.; The result in mitochondrial maturation, functional electrophysiology, and oxidative metabolism is progressive.	✓ ✓	Regular calcium transient activities, and peak amplitude, and action potential frequencies with proper excitation-contraction coupling, and depolarization, and repolarization.	Cardiogenesis; Related cardiac disease modeling (proof-of-concept demonstration by ondansetron)	24
2021 Song et al.	Cardiac mesoderm cell- or cardiomyocyte-derived-COs (CMC or CM-COs)	H	hPSCs aggregates; Poly-HEMA	CHIR, IWP2; CMC-COs: B27- Ins; CM-COs: B27-Vitamin A.	CMC-COs exhibited more organized sarcomeres, mitochondria, well-arranged t-tubules, and evenly distributed intercalated discs compared to CM-COs.	✗ ✓	CMC-COs exhibited more mature electrophysiological characteristics characterized by faster motion vector speed, reduced beats per minute, increased peak-to-peak duration, prolonged	Cardiogenesis; Related genes and mechanisms validation.	25

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Table 1 (continued)

Year& Authors	Model	Sp. Cell types& Scaffold	Signal stimulus	Characteristics (Morphological structure & cellular composition)	Cardiac chamber & Spontaneous beating	Electrophysiological properties	Application	Ref.
2022 Ergir et al.	Human organotypic cardiac microtissues (hOCMTs)/human cardiac organoids (hCOs).	H hPSCs aggregates; ✕	Monolayer cardiac differentiation: Biphasic WNT (activate/inhibit) signal, Ins addition; CHIR, IWP2, B-27 minus Ins, B-27 Ins. hOCMT generation and maintenance: B-27 Ins.	Two-dimensional monolayer culture spontaneously differentiated into multiple cell types (cardiomyocytes, fibroblasts/epicardial cells, and ECs); 3D aggregated cultures form hOCMTs, showing improved cardiac ultrastructural organization and metabolic maturation. Embedded human iPSCs in 10% Matrigel on a shaker (60 –70 rpm) in ultra-low attachment petri dishes; Spontaneously beating, forming myocardium maturation and epicardium with distribution of cardiac-specific fibroblasts and SMCs; Mimicry of cardiac chamber structures, including epicardium/myocardium, atrium/ventricle-similar areas, vascularization, and mechanical/electrophysiological properties.	✕ ✓	APD50 and APD90, and repolarization, more negative resting membrane potential, and a high proportion of ventricular-like APs. Sustained spontaneous beating for over 100 days; No other electrophysiological properties were assessed except for contractility.	Cardiogenesis; Disease modeling (chemotherapy-induced cardiotoxicity)	26
2022 Lee et al.	Chamber-formed human heart organoids (CF-hHOs)	H hiPSCs aggregates; Matrigel	GSK-3 and WNT signals; 10% Matrigel, CHIR, C59, B-27 Ins (–).		✓ ✓	Showed field potential (FP) changes similar to electrocardiogram (ECG), including depolarization and repolarization processes; Regular and dynamic beating pattern of systolic/diastolic coupling, and the beating rate decreased significantly as maturation progressed.	Cardiogenesis; <i>In vivo</i> transplantation	27

2022 Branco et al.	Epicardium-myocardium organoids (EMOs).	H	hiPSC-derived lateral plate mesoderm progenitors; ✗	WNT (activate/inhibit) signal; BMP4, RA, CHIR; EMO establishment: re-aggregation and co-culture of PE/STM/PEH organoids and ventricle myocardium aggregate (1:9).	Generated a self-organized multilineage organoid with PE/STM- and PFH diverticulum-like cells, resembling early embryonic structures.	✓ ✓	Shown spontaneous calcium transients before and after drugs (isoproterenol, E-4031, and verapamil) stimulation.	Cardiogenesis; Pathophysiological mechanism	28
2023 Meier et al.	Epicardioid	H	hPSCs aggregates; Collagen I	WNT/Activin A/BMP signal axis, RA signal; CHIR, BMP4, Activin A, LY294002, Ins, IWP2, RA.	Generated RA-dependent self-organizing epicardioids that exhibit morphological, molecular, and functional patterning of the left ventricular wall; RA addition promotes earlier spontaneous beating, forms a dense inner core of cardiomyocytes, and a thick ventricular epicardium envelope.	✗ ✓	The subepicardial layer showed shorter durations to 50% and 90% peak decay and repolarization than the inner myocardium.	Cardiogenesis; Disease modeling (left ventricular hypertrophy and congenital myocardial fibrosis)	29
2025 Wang et al.	Human epicardial organoid	H	hPSCs aggregates; Matrigel	WNT/Activin A/BMP signal axis, RA signal, bFGF TGFβ; CHIR, BMP4, Activin A, LY294002, Ins, IWP2, SB431542, RA, LY294002, IMDM, F12 NUT-MIX, transferrin, monothioglycerol, PD-173074.	Spontaneously formed a compartment structure, similar to the epicardial layer formation; Transformed into cells with mesenchymal characteristics, further differentiated into smooth muscle cells, and secreted a variety of extracellular matrix (ECM) molecules; Possessed paracrine function and migratory ability, integrated into the mouse heart, and survived.	✓ ✗	Sustained spontaneous beating; No other electrophysiological properties were assessed except for contractility.	Cardiogenesis; Regeneration potential	30
2021 Silva et al.	Human multilineage COs	H	hiPSCs-derived mesoderm progenitors aggregates; ✗	Biphasic WNT (activate/inhibit) signal; CHIR, IWP2, ascorbic acid.	Recapitulated cooperative cardiac core and gut-like tube with extensive cellular organization; The endoderm tissue (gut/intestine) in the	✓ ✓	Exhibited mature spontaneous calcium activity over time, including higher amplitude, maximum rate of rise, and rate of fall, as well as the	Cardiogenesis; Tissue crosstalk mechanisms	31

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Table 1 (continued)

Year& Authors	Model	Sp.	Cell types& Scaffold	Signal stimulus	Characteristics (Morphological structure & cellular composition)	Cardiac chamber & Spontaneous beating	Electrophysiological properties	Application	Ref.
					multilineage COs contributed to cardiomyocyte expansion, compartmentalization, enrichment of atrial/ nodal cells, myocardial compaction, and fetal- like functional maturation.		response to electrical stimulation up to 8Hz.		
2021 Rossi et al.	Gastruloids	M	mESCs aggregates; ✗	CHIR, N2B27, bFGF, VEGF, L-ascorbic acid.	Self-organized mESCs facilitate the generation of cardiovascular precursor cells, leading to the specification of the first heart field (FHF) and second heart field (SHF); Supported the cardiac crescent-like structure and early cardiac tube- like structures formation, and reproduced the development of the cardiac crescent and foregut endoderm.	✗ ✓	Showed calcium handling properties similar to functional fetal tissue, including rhythmicity and electrical excitability of calcium transients; Pharmacologic interventions (calcium channel blockers and β-adrenergic agonists) could modulate the calcium transients.	Cardiogenesis;	32
2022 Olmsted et al.	Elongating multilineage organized gastruloids- derived cardiogenesis (EMLOC)	H	hiPSCs aggregates; ✗	N2B27, CHIR, FGF2, B27, IGF1, HGF, VEGF, ascorbic acid, Y27632.	Interconnected neurocardiac lineages contained co- developing central and peripheral neurons and trunk mesoderm for cardiogenesis and neurogenesis; Contained heart tube formation and specialization, cardiomyocytes differentiation, ventricular wall morphogenesis, epicardium, chamber-	✓ ✓	Spontaneous contractility and calcium transient frequency.	Cardiogenesis;	15

2024 Kostina et al.	Human neural crest- heart anlage (hNCHA)	H	hiPSC/iPSC-L1- mCherry aggregates; ✕	For heart organoid: Three-step WNT signaling axis (activate/ inhibit/activate), BMP signal axis; CHIR, BMP4, Activin A, Wnt-C59; For neural crest cells differentiation: N-2 supplement, CHIR99021, SB431542.	like structures, and outflow tract (OFT) formation, and exhibited neuro-cardiac interconnections. The size and morphology are similar to those of early developing human heart tissue; Contains multiple cell types, including cardiomyocytes, neural spine cells, glial cells, and ECs, and has a certain organizational hierarchy and structure; Supports a variety of neural functions, such as neuronal synaptic connections and signal transmission, and parasympathetic innervation mimics.	✕ ✓	Rhythmic contraction. Neural crest cell derivatives in hNCHA promote the maturation of the cardiac conduction system.	Neural crest cells -related cardiogenesis and CHD; High-throughput drug screening	33
2024 Dardano et al.	Blood-generating human pluripotent stem cell-derived heart- forming organoids (BG-HFOs)	H	hPSCs aggregates; Matrigel	Improved based on the scheme by Drakhlis et al. ²² with haemato- endothelial-driving growth factors: BMP4, bFGF, VEGF, SCF, IL- 11, EPO, IGFI, IL-6, TPO, FLT3, IL-3, SHH.	Highly similar to the first weeks of native human hematopoiesis, including PE/STN, various endothelium subtypes (VE, AE, HE, and HPCs), and the cluster of early primitive progenitors, megakaryocytes, erythroid derivatives, macrophages, and monocytes; In the whole organoid, the proportion of AE/ HE-4%, VE-8%, HPCs and mature hematopoietic derivatives-14%; ECs are found throughout, while HE, HPCs, and mature hematopoietic	✕ ✓	Shown immature ventricular-like low upstroke velocity, decreased typical plateau phase of action potential, and the rate of depolarization.	Cardiogenesis; Hematogenesis	34

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Table 1 (continued)

Year& Authors	Model	Sp. Cell types& Scaffold	Signal stimulus	Characteristics (Morphological structure & cellular composition)	Cardiac chamber & Spontaneous beating properties	Application	Ref.
2022 Liang et al.	Vascularized cardiac organoids	H hPSCs aggregates; iMatrx-511	WNT signaling axis, VEGF signal; CHIR, Wnt-C59, VEGF, Ins.	derivatives are exclusive to the outer layer; myeloid cells (monocytes and macrophages) are present in the outer layer and surrounding Matrigel. Contained 19% cardiac ECs and 48% cardiomyocytes; Exhibited more defined chamber-like structures and a more mature beating pattern compared to cardiac organoids.	Exhibited contractility similar to that of the embryonic heart, as well as functional membrane potentials.	Cardiogenesis; Chemotherapy drug-induced cardiotoxicity screening	35
2023 Voges et al.	Human vascular cardiac organoids (hVCOs)	H hPSCs differentiation; Collagen I and Matrigel	hVCO fabrication: SU-8 photolithography and polydimethylsiloxane (PDMS) molding; 20% vascular cells and 80% cardiomyocytes/fibroblasts; collagen I and Matrigel; B-27 Ins (-), L-ascorbic acid, aprotinin, B-27 Ins, Ins, FGF-2, PDGF-BB.	One-step differentiation for hVCOs underscores the critical role of endothelial cells in COs maturation; Optimized conditions support robust ECs in hCOs and promote the generation and self-sorting of multiple cardiac cell populations, including cardiomyocytes, fibroblasts, PEs, and epicardial cells.	Stronger contractility; No other electrophysiological properties were assessed.	Cardiogenesis; Disease modeling (Inflammation-induced cardiac dysfunction); Drug toxicity test	36
2024 Yang et al.	Vaschamcardioids (vcCOs).	H hiPSCs aggregates; Matrigel	Vascular spheres: CHIR99021, BMP4, FGF2, VEGFA; CHIR99021, Wnt-C59, L-ascorbic acid 2-phosphate, D,L-lactate; vcCOs; Thiazovivin, FGF2, VEGFA.	Displayed a vascularized and chamber-like structure, containing cardiomyocytes, ECs, fibroblasts, cardiac precursor cells, neuronal cells, and mesenchymal cells.	Showed typical membrane potential changes and action potentials; Rhythmic contraction and calcium transient capacity; The vcCOs had a 90% spontaneous beating ratio.	Cardiogenesis; Cryoinjury, Drug evaluation (captopril treatment)	37

2022 Feng et al.	Heart organoids (HOs)	H	hiPSCs aggregates; Poly (methacrylate)	WNT signaling; RA signal at different times and concentrations; B-27 minus Ins, CHIR, C59, B27, RA.	Two differentiation protocols oriented to individually generate atrial and ventricular organoids; Identified the core of RA in developmental distinction between atrial and ventricular lineages; Established an integrating HO, computational analysis to study heart development in normal and disease states.	✓ ✓	RA ⁻ and RA ⁺ organoids have typical atrial and ventricular action potentials, respectively; Shorter APD50, and APD75, APD90, and higher beating rates in the RA ⁺ organoids.	Cardiogenesis; Disease modeling (CHD carrying a point mutation in the NKX2-5, Ebstein's anomaly)	38
2023 Schmidt et al.	Human cardioid platform	H	hPSCs aggregates; ✗	Dual WNT and Activin signaling; Mesoderm induction: Activin A, BMP4; Cardiac mesoderm: BMP4, FGF2, Ins, C59, RA, SB431542, XAV939; Atria chamber: RA, FGF2, LDN-193189, LY-411575, T3 hormone, Ins; Ventricular maturation: Ins, PD0325901, SB203580, L-lactate, glucose, vitamin B12, biotin, creatine, taurine, L-carnitine, L-ascorbic acid, B-27; ECs differentiation: FGF-2, LY294002, Activin A, BMP4, Ins, CHIR, VEGF, Forskolin; OFT cardioids EMT: VEGF165, TGFβ, FGF- 2, BMP4, Ins; SMCs differentiation: Ins, ascorbic acid, TGF-β, PDGF, bovine fibronectin, PDGF; Multi-chambered cardioids assemblage:	Simulated RV, LV, atrium, OFT, atrioventricular canal (AVC) organoids respectively, and were assembled to form multi-chamber cardioids; The multi-chamber cardioids mimic the native heart's coordinated contractions between chambers and allow for studying the impact of mutations, drugs, and environmental factors on specific regions.	✓ ✓	All types of organoids showed spontaneous contraction, but the amplitude and frequency of contraction varied. The LV organoids showed more frequent contraction, and the RV and OTF organoids contracted less frequently; LV organoids exhibited higher calcium transients and faster signal propagation speed; Each contraction of the combined multi- chamber cardioids originated typically from one compartment and then propagated through the entire multi- chambered cardioid, mimicking the conduction of electrical signals in the real heart, such as action potential propagation and repolarization	Cardiogenesis; Pathophysiologic mechanism (dissects genetic, teratogenic, and physiological defects)	39

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Table 1 (continued)

Year & Authors	Model	Sp. Cell types & Scaffold	Signal stimulus	Characteristics (Morphological structure & cellular composition)	Cardiac chamber & Spontaneous beating properties	Electrophysiological properties	Application	Ref.
				using molds, put atrium, left ventricle (LV), and right ventricle (RV) in order.		processes.		
Abbreviation: Sp. = species, Ref. = references, H = Human, ✓ = have, ✗ = do not have, hPSCs = human pluripotent stem cells, hiPSCs = human induced pluripotent stem cells, ECs = endothelial cells, SMCs = smooth muscle cells, vCMs = ventricular cardiomyocytes, aCMs = atrial cardiomyocytes, HCM = hypertrophic cardiomyopathy, RA = retinoic acid, EMT = epithelial-mesenchymal transition, CHD = congenital heart defect, ICD = intercalated disc, APs = action potentials, APD50 = action potential duration at 50%, APD75 = action potential duration at 75%, APD90 = action potential duration at 90%, PE = pro-epicardium, STM = septum transversum mesenchyme, posterior foregut/hepatic = PFH, VE = venous, AE = arterial, HE = hemogenic endothelium, HPCs = hematopoietic progenitor cells, mESCs = mouse embryonic stem cells.								

Their research showed that cardiac mesoderm cell-derived COs exhibited higher self-organizing capacity and differentiated into more mature ventricular-like cardiomyocytes²⁵. Thus, the high self-organizing capacity of cardiac mesoderm cells is crucial for generating mature hCOs. Moreover, Ergir et al.²⁶ reported that hiPSCs undergo mesodermal cardiomyocyte differentiation under monolayer culture conditions and achieve early beating by WNT signaling stimulation (activation/inhibition), followed by further differentiation into contractile cardiomyocytes and stromal cells under additional insulin (Fig. 1). These monolayer cardiac differentiated cells were subsequently cultured in a 3D aggregation, resulting in spontaneous differentiation into human organotypic cardiac microtissues comprising cardiomyocytes, fibroblasts/epicardial cells, and ECs²⁶. It exhibits superior cardiac ultrastructural organization, metabolic maturation (with more mitochondria and glycogen, metabolic gene clusters similar to adult hearts, and enhanced metabolic activity), and sustained spontaneous beating for over 100 days²⁶. These characteristics should be considered in the subsequent optimization of cardioid construction.

2.2. Cardioids containing the epicardium

The epicardium, which covers the heart tube in early embryonic development, serves as a barrier between the pericardial cavity and myocardium and provides signals and cell lineages for heart growth and repair⁴⁷⁻⁵⁰. The aforementioned construction methods, such as those by Lewis-Israeli et al.²³, Lee et al.²⁷, and Ergir et al.²⁶, have shown the emergence of epicardial cells or the epicardium but remain immature in both structure and function. Hofbauer et al.¹⁴ did not observe epicardial cells within their cardioids; however, they developed an epicardial aggregate that spontaneously spread over the cardioids (Fig. 2), interacted with the myocardial cell layer, and migrated inward for differentiation¹⁴. And this process occurred in the absence of exogenous TGF β , FGF, or PDGF signals¹⁴. However, the need for artificially supplemented epicardial aggregates detracts from the self-organization advantages. Branco and colleagues²⁸ used hiPSC-derived lateral plate mesoderm progenitors to recreate multi-lineage pro-epicardium/foregut organoids that co-emerge pro-epicardium, septum transversum mesenchyme, and liver bud (Fig. 3). By supplementing with BMP4, CHIR, and Retinoic acid (RA), they generated WT1⁺ cells, which were co-cultured with cardiomyocyte aggregates to form self-organized heart organoids (Fig. 1). These organoids feature an epicardium-like layer surrounding the myocardium-like tissue and reproduce myocardium-epicardium interactions²⁸. Their advantage is in mimicking the early stages of pro-epicardium development and the morphology of early embryonic structures, including pro-epicardium, septum transversum mesenchyme, and anterior foregut. Afterwards, Meier et al.²⁹ developed an epicardioid model by modulating WNT/Activin A/BMP/RA signals in hPSCs embedded in collagen I. RA signaling is crucial for distinguishing atrial and ventricular lineages at the cardiac mesoderm stage (Fig. 1)⁵¹⁻⁵³. Additionally, RA was essential for efficient epicardium formation, promoting earlier spontaneous beats, dense cardiomyocyte packing, and a thick ventricular epicardial envelope in these epicardioids²⁹. Therefore, this model replicates the embryonic epicardium, providing progenitors for cardiac lineages (e.g., fibroblasts, smooth muscle cells, and cardiomyocytes) and paracrine signals that drive myocardial compaction and maturation²⁹. Recently, Wang et al.³⁰ successfully generated other epicardial

Table 2 Construction methods for self-organizing cardioids and their advantages and limitations.

No.	Summary construction scheme	Advantage	Limitation	Ref.
Basic scheme of constructing human self-organizing cardioids				
1	1. Embed hPSC aggregates in Matrigel. 2. Direct cardiac differentiation through biphasic modulation of the WNT pathway using CHIR99021 and IWP2. 3. In suspension culture to form HFOs.	1. Recapitulates key features of early heart and foregut development, including myocardial, endocardial-like, and foregut endoderm tissues. 2. Provides an <i>in vitro</i> model for studying gene-knockout phenotypes. <i>NKX2-5</i> KO HFOs showed cardiac malformations similar to those of mouse models.	1. It only models the early stages of heart, and cannot fully recapitulate subsequent processes. 2. Insufficient to maintain early structure and maturation.	22
2	1. Temporal control of key cardiogenic signaling pathways (Activin, BMP, FGF, RA, and WNT) in hPSCs to specify mesoderm, cardiac mesoderm, and cardiomyocyte progenitors. 2. Addition of laminins 521/511 before mesoderm induction. 3. Without exogenous ECM in high-throughput non-adherent 3D culture.	1. Recapitulates key features of early heart development, including chamber-like morphogenesis and lineage identity. 2. Allows for the study of congenital heart defects and heart disease mechanisms. 3. Explore the complex developmental mechanisms in an organ-specific context.	1. Limiting the modeling of most cardiac defects occurring later. 2. The complexity of <i>in vivo</i> heart development, like interactions with other germ layers, cannot be reproduced. 3. Impossible to faithfully model regenerative fetal and neonatal <i>in vivo</i> injury responses.	14
3	1. Use hPSCs to generate cardiac organoids through a three-step WNT signaling modulation strategy by chemical inhibitors and growth factor stimulation. 2. The process involves the formation of embryoid bodies followed by specific induction steps to promote cardiac differentiation.	1. Recapitulates key features of early heart development, including chamber-like morphogenesis, atrioventricular specification, and vascularization. 2. High structural and cellular complexity, including cardiomyocytes, epicardial cells, endocardial cells, cardiac fibroblasts, and ECs. 3. Exhibits robust beating, electrophysiological activity, and calcium transients. 4. Provides a pregestational diabetes-induced congenital heart defect model.	1. Might deviate from the normal developmental path in limiting the relevance in long-term culture. 2. Limited to fully recapitulate heart development compared to mouse models. 3. Needed further improvements to better mimic morphological and anatomical features, and to induce effective vascular networks.	23
4	Optimized the above scheme²³ by the same lab to form the EMM2/1 scheme. 1. Utilizes a combination of two media formulations (EMM2 and EMM1) from day 20 to day 30 of culture. 2. EMM2 media (Days 20–26) includes components like RPMI 1640 medium, B27 supplement, fatty acids (palmitate-BSA, oleate-BSA, linoleate-BSA), L-carnitine, T3 hormone, ascorbic acid, glucose, and IGF1. 3. From day 26 onwards, uses EMM1 media (similar base composition but without IGF1). 4. Aims to mimic <i>in utero</i> heart development conditions more closely than others.	1. Closely recapitulates human first-trimester heart development, including anterior-posterior heart tube patterning. High similarity to human embryonic hearts at 6–7 gestational weeks. 2. Produces heart organoids with high complexity and anatomical relevance, including distinct atrial and ventricular chambers, a pro-epicardial organ, and diverse cardiac cell types. 3. Promotes mitochondrial and metabolic maturation and enhances electrophysiological maturation. 4. Provides a powerful tool for disease modeling and pharmacological studies.	1. Seemingly has negative effects on organoid vascularization. 2. Lack of circulation. More anatomical events need to be modeled, including OFT and AVC formation, heart looping, and chamber septation. 3. Needs to increase the physiological relevance of the organoids.	24
5	1. Induce differentiation of hPSCs into CMCs and cardiomyocytes (CMs) 2. Isolate CMCs or CMs into single-cell suspensions and seed in poly-HEMA-coated dishes to undergo spontaneous aggregation into COs.	1. CMC-COs exhibit more mature structural and functional characteristics, including organized sarcomeres, mitochondria, t-tubules, and intercalated discs. 2. CMCs have a high self-organization capacity.	1. Long culture Period: Approximately 30 days from cell differentiation to organoid maturation. 2. High technical requirements: Precise control of cell density and filtering processes is necessary.	25

(continued on next page)

Table 2 (continued)

No.	Summary construction scheme	Advantage	Limitation	Ref.
	3. Optimize culture conditions and filter cells to obtain uniformly sized (70–100 μm) COs.	3. CMC-COs—for disease modeling and drug screening.		
6	1. Induce differentiation of hiPSCs in 2D culture towards cardiovascular lineage. 2. Followed by further aggregation in low-attachment culture dishes to generate hOCMTs.	1. Exhibited self-organization and functionality over an extended cultural time. 2. Displayed improved cardiac specification, survival, and metabolic maturation. 4. Responded to cardioactive and cardiotoxic drugs, suitable for studying chemotherapy-induced cardiotoxicity.	1. Still displayed features of the fetal rather than the adult heart. 3. Cellular Heterogeneity.	26
7	1. Ventricular-lineage heart organoids: Established a three-dimensional differentiation protocol by sequentially modulating the WNT signaling pathway, allowing differentiation of hiPSCs into cardiac lineages in organoid and monolayer systems. 2. Atrial-lineage heart organoids: Modified the differentiation workflow by treating with RA at cardiac mesoderm and progenitor stages.	1. Establishes the chamber defect model. 2. The optimized protocol generates the first atrial and ventricular lineage organoids, suitable for the study of atrial and ventricular lineage developmental heart defects.	1. Immaturity of cardiomyocytes. 2. Structural inconsistency and heterogeneity: Reveals three categories of organoids—intact, holes, and cavities. 3. The distribution of fibroblasts was uneven. 4. The current system does not model tricuspid valve defects associated with Ebstein's anomaly.	38
Cardioids containing the epicardium				
8	1. Induce hiPSCs to differentiate into hHOs by using a self-organization protocol based on Matrigel in ultra-low attachment dishes. 2. The protocol involves sequential modulation of WNT signaling and small molecule compounds to promote cardiac differentiation and chamber formation.	1. Spontaneous organization into chamber-like structures without external molds or physical stimulation. 2. Closely mimic the structural and functional characteristics of the human heart, including chamber formation, atrial/ventricular-like regions, and epicardium/myocardium organization. 3. Allow the multiple organoid generation in a single dish. 4. Exhibit vascularization and drug responsiveness.	1. Still exhibit fetal characteristics rather than adult hearts. 2. Lack of endocardium 3. Observed vascularization, while forming a complete vascular network.	27
9	1. Differentiate hPSCs into pro-epicardium and septum transversum mesenchyme organoids by modulating WNT, BMP, and RA signaling pathways. 2. Co-cultured these organoids with cardiomyocytes to generate EMOs.	1. Self-organized into distinct layers of epicardial-like and myocardial-like tissues, reproducing the role of epicardium. 2. Useful for studying heart development, modeling congenital heart diseases, and drug screening.	1. Require extended periods to achieve functional maturation. 2. Structural changes may occur with prolonged incubation. 3. Cannot fully replicate the complexity of native heart tissue.	28
10	1. Generate epicardioids from hPSCs through WNT, Activin A, BMP4, and bFGF signaling pathways modulation, with the addition of RA for specific stages. 2. The spheroids' formation is followed by embedding in collagen I.	1. Form a structured layer similar to native cardiac tissue, including ventricular myocardium and epicardium. 2. Reproducing important functions of the epicardium. 3. Interactions between different types of cardiomyocytes could be studied, as well as complex cardiac disease models.	1. Requires extended periods for differentiation and maturation, while the structure of epicardioids might change in prolonged culture. 2. Could not fully replicate the complexity of native cardiac tissue, especially vascularization and innervation.	29
11	1. Induce hESCs and iPSCs to form spheroids using a medium containing BMP4, bFGF, CHIR99021, LY294002, and Activin A. 2. Epicardial specification: From day 3.5 to day 6.5, culture spheroids in a medium containing BMP4, IWP2, SB431542, BMS, and PD-173074. Subsequently, use	1. Resemble human fetal epicardium in terms of EMT and paracrine ability. 2. Exhibits rapid migration capabilities, can integrate into mouse hearts, and efficiently generates cells with cardiomyocyte-like characteristics.	1. Lack of autonomous contractions. 2. No cardiomyocytes. 3. Cannot fully replicate the complexity of the entire heart, such as the electrical activity. 4. When transplanted into immunocompromised mice, there is a risk	30

Table 2 (continued)

No.	Summary construction scheme	Advantage	Limitation	Ref.
	a medium with BMP4, CHIR99021, and RA until day 9.5. 3. Maintenance of epicardium from day 9.5 onwards with insulin and SB-431542. 4. EMT induction: On day 9.5, change the medium for TGF β followed by bFGF.	3. Forms a chamber structure containing multiple cell types, including smooth muscle cells and fibroblasts. 4. Paracrine function and EMT capability: Secretes extracellular matrix and growth factors, mimicking the subepicardial space environment. 5. Useful for both <i>in vitro</i> studies and <i>in vivo</i> transplantation experiments.	of immune rejection in clinical applications. 5. Long-term culture of organoids may lead to changes in cell characteristics and functions.	
Cardioids of multi-layer/organ coordinated development				
12	1. Aggregation of hiPSC-derived mesendoderm progenitors supports multilineage differentiation. 2. Culture in additional ascorbic acid-supplemented media promotes the co-development of cardiac (mesoderm) and gut (endoderm) tissue. 3. Maintain for up to 1 year for the co-emergence and maturation of cardiac and gut tissues.	1. Simulate the co-development of cardiac and gut tissues, mimicking the interaction during embryogenesis. 2. Exhibit advanced structural complexity, including epicardial layers, myocardial compaction, and gut-like epithelial structures. 4. Providing a stable platform <i>via</i> remaining viable and functional for over 1 year in culture.	1. Requires extended periods for differentiation and maturation. 2. Difficult to obtain high-quality imaging of intact spheroids at the late stage of culture due to their large size.	31
13	1. Culture mESCs in N2B27 medium supplemented with CHIR99021, bFGF, VEGF, and L-ascorbic acid to form gastruloids. 2. Maintains culture for up to 168 h to observe the development of cardiac and vascular structures.	1. Mimics early heart development, including the formation of FHF and SHF progenitors and the cardiac crescent. 2. Support the co-development of cardiac, vascular, and endodermal tissues, mimicking the interactions. 3. Generates multiple cell types, including cardiovascular progenitors, ECs, and cardiomyocytes. 4. Exhibit functional properties comparable to early embryonic, such as calcium handling and rhythmic contractions.	1. Different cell lines might show variability in differentiation efficiency and cell-type composition, which can affect the reproducibility. 2. The limited lifespan in the gastruloids restricts long-term studies of cardiac development and maturation.	32
14	1. Induce hiPSCs for elongating multilineage organized (EMLO) gastruloids by introducing angiogenic and pro-cardiogenic factors (VEGF and ascorbic acid). 2. Involve a 2D induction phase followed by aggregation in shaking cultures, with specific growth factors added at different stages.	1. Provides a multi-lineage context, including cardiomyocytes, neurons, ECs, fibroblasts, and other cell types, mimicking cardiogenesis and neurogenesis. 2. Displays cardiogenic compartments, recapitulating key developmental features such as heart tube and OFT formation, and chamber-like structures 3. Achieves neurogenesis and integrates neurons with cardiac tissue.	1. Need further functional validation for the neuron functionality and the interaction with cardiomyocytes. 2. Challenges in reproducibility due to a complex protocol with multiple steps and specific growth factor additions.	15
15	1. The human neural crest-heart assembloids (hNCHAs) are created by integrating hiPSC-derived neural crest cells into human heart organoids to form neural crest-heart assembloids (hNCHAs). 2. Engineered the hiPSC line to stably express mCherry for easy detection and monitoring of neural crest cells.	1. Closely mimics the <i>in vivo</i> migration of cardiac neural crest cells (NCCs) into the embryonic heart and their differentiation into major cardiac NCC derivatives. 2. Recapitulates the majority of transcriptional features reported for NCC development and their derivatives.	1. Does not replicate the heart looping and complete maturation of the OFT. 2. Lacks trunk NCCs, which are essential for forming cardiac sympathetic innervation. 3. Absents crucial physiological factors such as blood flow and mechanical strain.	33

(continued on next page)

Table 2 (continued)

No.	Summary construction scheme	Advantage	Limitation	Ref.
16	3. Combines these neurospheres with heart organoids (on the 17th day by previous protocols ²³) to form hNCHAs.	3. Offers a powerful tool for studying NCC-driven heart development and its associated processes.	4. Does not incorporate interactions with other cell types and tissues of endodermal origin.	34
	1. Modifies the previously established HFOs ²² to develop blood-generating heart-forming organoids. 2. Adds specific growth factors and cytokines at specific time points to stimulate hemogenic endothelium formation and hematopoiesis.	1. Provides a structured multi-tissue model, recapitulating the co-development of cardiac, endothelial, and hematopoietic tissues. 2. Includes various cell types such as cardiomyocytes, ECs (arterial and venous subtypes), hemogenic ECs, and hematopoietic progenitor cells with erythro-myeloid and lymphoid potential. 3. Allows functional analysis of contraction patterns, action potential colony-forming unit, and lymphoid potential. 4. Enhances the understanding of developmental mechanisms and interactions between different tissue types.	1. Limited hematopoietic potential. Although displaying erythro-myeloid and lymphoid potential, it lacks bona fide hematopoietic stem cell competence.	
The vascularization of cardioids				
17	1. Utilize hPSCs to develop vascularized cardiac organoids with chamber-like structures by modulating the WNT signaling pathway. 2. Involved temporal activation and inhibition of WNT signaling using CHIR99021 and Wnt-C59, along with VEGF and TGFβ inhibitor to form endothelial-bound chambers.	1. Exhibits chamber-like structures and a more mature membrane potential compared to organoids composed solely of cardiomyocytes. 2. Enhanced functional maturity. 3. Demonstrates a better response to toxic drugs, making it more effective for studying cardiotoxicity and drug screening. 4. Incorporates multiple cell types, including cardiomyocytes and endothelial cells.	1. Still lacks the full complexity of the native heart, including other cell types and extracellular matrix components. 2. Immaturity of derived ECs compared to primary human cardiac microvascular ECs.	35
18	1. Incorporates vascular cells into hPSCs-derived cardiac organoids. 2. Optimizes protocols to generate multiple cardiac cell types, including cardiomyocytes, fibroblasts, epicardial cells, endothelial cells, and pericytes, which self-organize into <i>in vivo</i> -like structures. 3. Combines vascular cells with cardiac cells in a specific ratio (20% vascular cells to 80% cardiac cells) and cultures in a serum-free maturation medium.	1. Enhanced functionality by the addition of vascular cells in contractile force and maturation markers. 2. Better suited for modeling inflammation-induced cardiac dysfunction. 3. Contains a diverse array of cell types, including ECs, pericytes, fibroblasts, and cardiomyocytes. 4. Reveals paracrine signaling (PDGFB and LAMA5) blessing in the constructions.	1. Limits the ability to study cellular population interactions. 2. Difficult to achieve precise cell composition perturbations because the single differentiation step produces all cell types. 3. Vascular cells lack full structural maturity 4. Not readily dissociated, thereby imposing limitations on certain analytical methodologies.	36
19	1. A three-step method to generate vascularized and chambered cardiac organoids from hiPSCs. 2. Induce hiPSCs to form vascular spheres. 3. Envelopes the vascular spheres with hiPSCs-derived cardiomyocytes. 4. Induce the central vascular cells to migrate outward in response to a VEGF gradient, leading to the formation of vascularized and chambered cardiac organoids.	1. High Reproducibility: Generated vcCOs with over 90% spontaneous beating organoids consistently. 2. Contains multiple cell types, including cardiomyocytes, ECs, fibroblasts, cardiac progenitor cells, neurocytes, and mesenchymal cells. 3. Recapitulate processes of cardiac injury and fibrosis, suitable for studying cardiovascular diseases, as well as drug efficacy and toxicity testing.	1. Lack of immune cells, not conducive to the detection of the early response to myocardial infarction and the subsequent fibrotic response. 2. The maturity of cardiomyocytes is intermediate between fetal and adult hearts. 3. The method involves multiple steps and specific culture conditions, posing challenges for reproducibility and scalability.	37
The assembled typical multi-chamber cardioids				

Table 2 (continued)

No.	Summary construction scheme	Advantage	Limitation	Ref.
20	1. The construction steps of chamber-like cardioids refer to the method in No.2¹⁴. 2. Generates separate epicardial aggregates from the same hPSCs. 3. The chamber-like cardioids and epicardial aggregates are cultured together in media without exogenous TGFβ, FGF, and PDGF. 4. Within 2–7 days, epicardial cells spread on top of the cardioids. 5. After 7 days, without additional growth factors, epicardial cells interacted with the CM layer, migrated into it, and underwent differentiation.	1. Allows for studying the interactions between epicardial cells and cardioids in the absence of external signals. 2. Provides a more complete model of heart development by including the third major cardiac lineage.	1. The complexity of the co-culture system, the potential variability in the extent of epicardial integration and differentiation. 2. Needs for precise control of the conditions to ensure proper interactions between the two components.	14
21	1. Leverages both 2D and 3D differentiation to efficiently generate progenitor subsets with distinct first, anterior, and posterior second heart field identities. 2. Induces mesoderm first, followed by specific patterning stages using a combination of signaling inhibitors and activators (WNT, Nodal/Activin, BMP, and RA). 3. Carefully controlling the signaling environment during mesoderm induction, and specifying patterning, progenitors from the FHF, anterior second heart field (aSHF), and posterior second heart field (pSHF). 4. Dissociates and reaggregates progenitors in specific conditions to form cardioids. It includes precise cell counting and seeding density optimization. 5. Generates different cardioid subtypes (LV, RV, atrial, OFT, AVC) by adjusting the concentrations of specific signaling molecules and growth factors during the patterning stages. 6. Uses polydimethylsiloxane (PDMS) molds to form multi-chamber cardioids.	1. Recapitulates the development of all major embryonic heart compartments, including right and left ventricles, atria, OFT, and AVC, and provides a more complete model of human heart development compared to others. 2. Highly efficient and reproducible, compatible with multiple cell lines, enabling high-throughput screening with various readouts. 3. Multi-compartment Integration: Allows for the study of interactions between different heart compartments. 4. Provides a human-specific model that overcomes limitations associated with animal models. 5. Adapts for various applications, including drug testing and the study of environmental effects on heart development.	1. Primarily focuses on the early stages of heart development, but does not fully represent later stages and processes. 2. Not incorporate processes like aSHF/pSHF progenitor migration and heart looping. 3. Not fully replicate the complex three-dimensional structure and organization of the native human heart. 4. Requires precise control of multiple signaling pathways and culture conditions. 5. Cardiomyocytes in the cardioids may not fully mature like adult cardiomyocytes, potentially affecting the accuracy of functional studies.	39

organoids from hPSCs by modulating WNT, BMP, and RA signaling in a three-step protocol. In contrast to the approach by Meier et al.²⁹, their team induced epithelial-mesenchymal transition through treatment with TGF β and bFGF, enabling the cells to acquire a mesenchymal state and subsequently differentiate into smooth muscle cells. The extracellular matrix (ECM) molecules secreted by this type of epicardial organoids closely resemble the ECM composition of the human subepicardial, including various collagens, fibronectin, and other components³⁰.

2.3. Cardioids of multi-layer/organ coordinated development

The heart organoids developed by Branco et al.²⁸ and Meier et al.²⁹, as mentioned earlier, effectively replicated myocardium–epicardium interactions, respectively. Furthermore, various multilineage iPSC-derived organoids have also partially recapitulated the paracrine regulation. Silva et al.³¹ described a such organoid of embryonic mesoderm (heart) and endoderm (gut/intestine) that develops cooperatively (Fig. 3). These organoids

sequentially express cardiac and gut master regulatory genes, with endoderm promoting cardiomyocyte expansion, atrial/nodal cardiomyocyte specification, myocardial compaction, and maintaining fetal-like architecture and function for over 100 days (Fig. 1)³¹. It does provide more insights into tissue crosstalk mechanisms, particularly in genetic diseases impacting both the heart and gut. Similarly, mouse ESCs-derived gastruloids have successfully mimicked early heart development, forming a cardiac crescent, closely resembling the human embryo^{32,54}. The cardiac crescent-like structure co-developed with tubular epithelial tissue, resembling the coordinated development of cardiac crescent and foregut endoderm, and sheds light on the complex interactions between cardiac precursors and surrounding tissues³². Additionally, a combined cardiogenesis and neurogenesis model was reported by optimizing interconnected neurocardiac lineages within a single organoid (Fig. 3)¹⁵. Based on hiPSC aggregates-derived elongating multilineage organized (EMLO) gastruloids containing co-developing neurons and trunk mesendoderm⁵⁵, Olmsted et al.¹⁵ replaced continuous HGF and IGF1 stimulation with

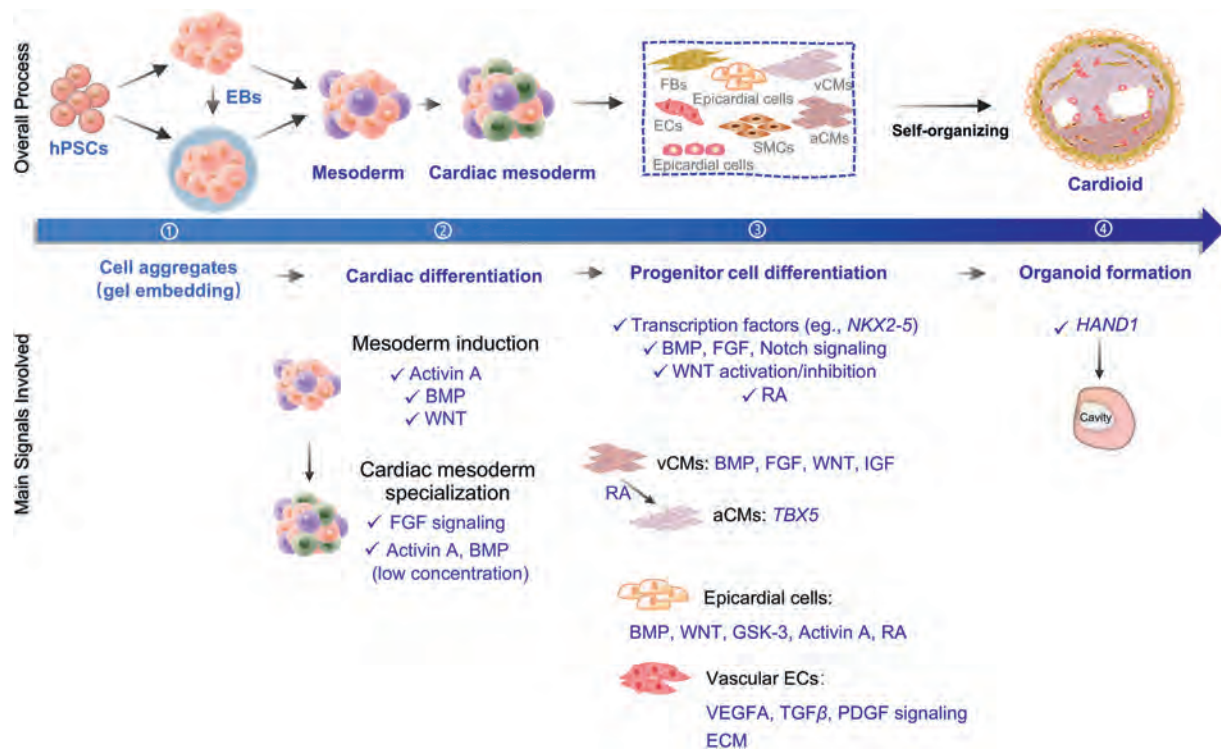


Figure 1 Schematic diagram of the current directly self-organizing cardiac organoids differentiation patterns. Human pluripotent stem cells (hPSCs) are utilized to aggregate with and without the scaffolds (such as Matrigel, iMatrx-511, poly-HEMA, Collagen I, etc.), and gradually develop embryonic bodies (EBs), mesoderm and cardiac mesoderm, by precise signal stimulation. Multiple types of cardiac cells will be differentiated through multiple signaling stimulations, and subsequently constructed into cardioids by a mixture of self-organizing or artificial integration. Induction from EBs into mesoderm necessitates the activation of Activin A, BMP, and WNT signaling; However, the specialization of cardiac mesoderm requires lower concentrations of Activin A and BMP in conjunction with FGF signaling stimulation. The differentiation and proliferation of cardiac lineage progenitor cells are regulated by key transcription factors, such as *NKX2-5*, and multiple signaling pathways, including BMP, FGF, Notch, RA, and WNT. Notably, the WNT signaling pathway requires precise bidirectional regulation through both activation and inhibition at specific temporal stages. The generation of CMs, in addition to the aforementioned regulations, might also necessitate the involvement of the insulin-like growth factor (IGF). Additionally, RA is crucial for the differentiation of aCMs, and the transcription factor *TBX5* plays an important role. Some studies have shown that the epicardial cells' differentiation also needs the support of BMP, Activin A, WNT, GSK-3, and RA. Immature vascularized branches composed of vascular ECs may be regulated by VEGFA, TGF β , PDGF, extracellular matrix (ECM) molecules, etc. In the self-organizing formation of cardioids, the transcription factor *Hand1* is the key to forming the cavity, which might be similar to the cardiac chamber. CM, cardiomyocyte; vCMs, ventricular cardiomyocytes; aCMs, atrial cardiomyocytes; FBs, fibroblasts; ECs, endothelial cells; SMCs, smooth muscle cells; RA, retinoic acid.

VEGFA and ascorbic acid to facilitate human cardiogenesis (EMLO-derived cardiogenesis). The key advantage of this model is its establishment of neuro-cardiac interconnections, mimicking an innervated heart. Kostina et al.³³ developed a novel human neural crest-heart assembloid (hNCHA) with autologous and developmentally relevant cardiac neural crest-derived tissues, with particular emphasis on the critical role of neural crest cells in cardiac development. It could effectively model the migration trajectories of neural crest cells within the human embryonic heart and differentiate into cardiac-related cell types, including parasympathetic neurons and mesenchymal cells³³. Moreover, hNCHA-derived neural crest cells are capable of establishing functional connections with cardiomyocytes, thereby facilitating the maturation of the cardiac conduction system³³. On the other side, Dardano et al.³⁴ developed blood-generating heart-forming organoids to model the co-development of the heart, vasculature, and foregut. This work built on their previous research by adding haemato-endothelial-driving growth factors (Table 1) at specific times and doses, promoting the coordinated development of the

dorsal aorta, hematopoiesis, and heart tissue^{22,34}. These modifications enable the organoids to mimic early human hematopoiesis, producing proepicardium/septum transversum mesenchyme, producing proepicardium/septum transversum mesenchyme, endothelial subtypes, early progenitors, megakaryocytes, erythroid cells, macrophages, and monocytes. This *in vitro* model offers valuable insights into hematopoiesis during cardiomyogenesis.

2.4. The vascularization of cardioids

Despite a high vascular branching point in the cardioids established by Lewis-Israeli et al.²³ without any additional steps, it falls short of meeting the requirements for a complete hCO structure. Liang et al.³⁵ highlighted that WNT signals regulated hPSCs differentiation into cardiac ECs and formed vascularized cardiac organoids with chamber-like structures. Moreover, Lee et al.²⁷ developed chamber-formed human heart organoids with microvascular networks that could be connected to the blood vessels of the mice and maintain beating and functionality after being transplanted into nude mice *in vivo*. In addition to vascularization, it exhibited

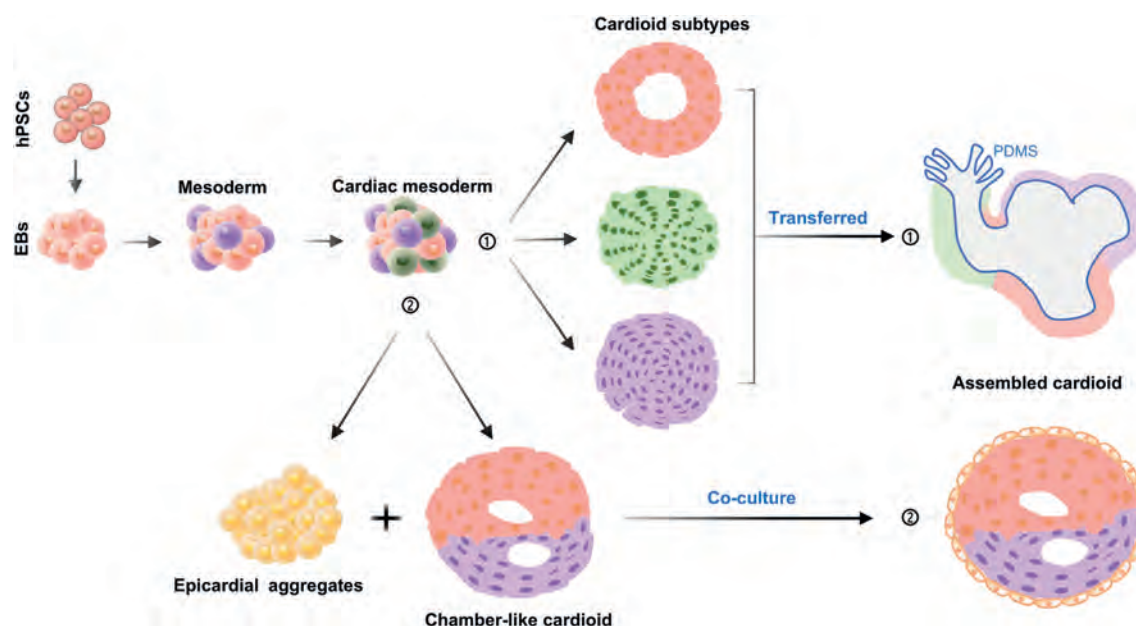


Figure 2 Schematic illustration of the assembled cardioids. The current methodologies for artificial integration of cardiac organoids can be categorized into two manners: ① Constructs multiple lineage-specific heart organoids separately, and they are transferred into the polydimethylsiloxane (PDMS), an embedding mold, to artificially form a multi-chamber cardioid; ② Generates a separate epicardial aggregate, and cultures together with the chamber-like self-organizing cardioid.

spontaneous beating, possessed gene expression profiles similar to those of human heart tissues, formed myocardium and epicardium with the distribution of cardiac-specific fibroblasts and smooth muscle cells, and displayed mechanical/electrophysiological characteristics. The procedure was accomplished by sequentially modulating GSK3 and WNT signaling after hiPSCs formed embryonic bodies (Fig. 1)²⁷. Voges et al.³⁶ demonstrated that vascular population cells within hCOs enhance myocardial contraction force, aligning with the roles of microvascular and coronary ECs in the heart^{36,56}. This highlights the significance of vascular cells in organoids. Vascular cells in hCOs stimulate platelet-derived PDGFR β secretion, increasing matrix deposition and enhancing contractile force, and play a crucial role in diastolic dysfunction caused by inflammatory factors³⁶. Additionally, Yang et al.³⁷ constructed vaschamcardioids (vcCOs) that exhibited a vascularized and chamber-like structure with spontaneous beating. Briefly, it is a three-step robust method that first induces hiPSCs to form vascular

spheres and cardiomyocytes separately, then combines them (with cardiomyocytes surrounding the vascular spheres), and finally vascular cell migration into the myocardial layer using VEGFA and FGF2 stimulation³⁷. While this method effectively mimics cardiac vascularization, it does not fully replicate key processes of heart organogenesis and components. Therefore, the development of fully vascularized cardioids requires further experimentation, potentially using the mentioned techniques.

2.5. The assembled typical multi-chamber cardioids

Although the aforementioned technologies generate chamber-like structures, precisely identifying specific anatomical sites remains challenging. Feng et al.³⁸ developed two differentiation protocols to generate atrial and ventricular organoids. RA-stimulation generated atrial-lineage organoids containing atrial cardiomyocytes, fibroblasts, and ECs, while the absence of RA led to

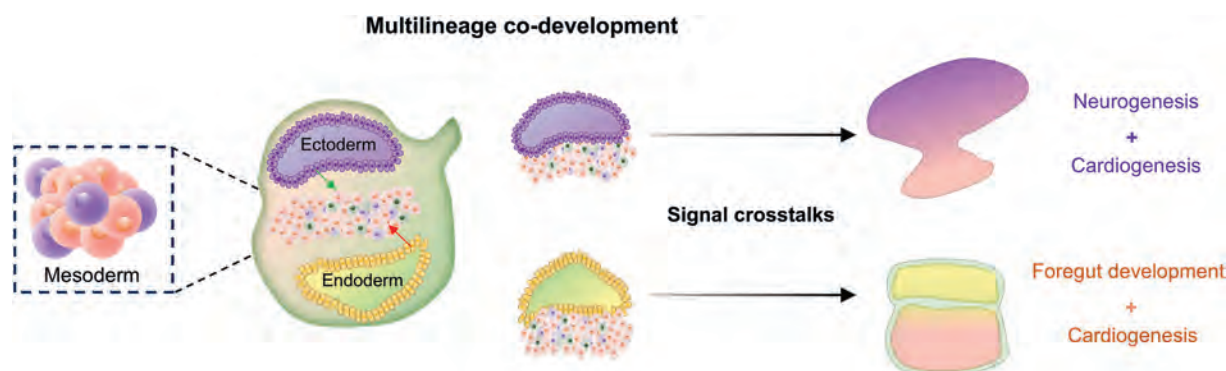


Figure 3 Multi-layer/organ coordinated development. The paracrine signals of adjacent germ layers (endoderm, ectoderm) more effectively facilitate heart development through intricate signal crosstalk mechanisms. Among current technologies, there are organoids that co-develop neural structures (endoderm) and the heart (mesoderm), as well as organoids representing the co-development of the foregut (ectoderm) and the heart.

ventricular-lineage organoids characterized by ventricular cardiomyocytes (Fig. 1)³⁸. It would be a significant breakthrough to organically integrate atrial and ventricular organoids, creating functional atrium-ventricle organoids. Mendjan's lab has successfully assembled a multi-chamber cardioid by optimizing WNT/BMP signaling to generate multiple organoids mimicking the right ventricle (RV), left ventricle (LV), atrium, outflow tract, and atrioventricular canal³⁹, building on their 2021 breakthrough¹⁴ (reagent details are given in Table 1). They efficiently induce anterior second heart field (aSHF) progenitor subset and inhibit first heart field (FHF) differentiation from mesoderm *via* dual inhibition of WNT and Activin signaling³⁹. Adding RA during the aSHF stage promotes posterior second heart field (pSHF) identity while inhibiting aSHF³⁹. This process is regulated by developmental signaling pathways like WNT, Activin, BMP, etc., at specific stages⁵⁷. The authors respectively generated different cardioids from aSHF and pSHF progenitors, and explored their specification potential along with FHF cardioids by adjusting RA concentrations. Higher RA dosages and C59 (both canonical and non-canonical WNT inhibition) promote aSHF specification to RV cardioids, whereas lack of exogenous RA and XAV-939 (inhibits only canonical WNT) stimulates the outflow tract (OFT) related gene expression within cardioids, and provokes the development

of OFT cardioids³⁹. In addition to forming atrial identity, pSHF were able to differentiate into the atrioventricular canal (AVC) cardioids by altering the mesodermal induction conditions to intermediate Activin and low WNT activation levels, then high exposure of the AVC region to BMP4³⁹. The multi-chamber cardioids were artificially integrated using polydimethylsiloxane (PDMS) molds (Fig. 2). It is one step closer to the native heart than others and has been used to explore electrophysiological signal propagation between chambers, as well as to dissect the defects in heart development.

2.6. Summary of constructing cardioids

Overall, the first step in the current construction of self-organizing cardiac organoids mainly involves the formation of embryonic bodies by aggregating hPSCs with/without biological scaffolds. Then, it sequentially differentiates into mesoderm/cardiac mesoderm and self-organizing specific cardiac lineage cells under multiple chemical stimuli. Additionally, signal stimulations from other germ layers are also essential for the organoid development. The final cardioids are formed by direct self-organizing assembly, or by additional artificial integration (Figs. 1 and 2). As an emerging technology, self-organizing cardiac organoids still

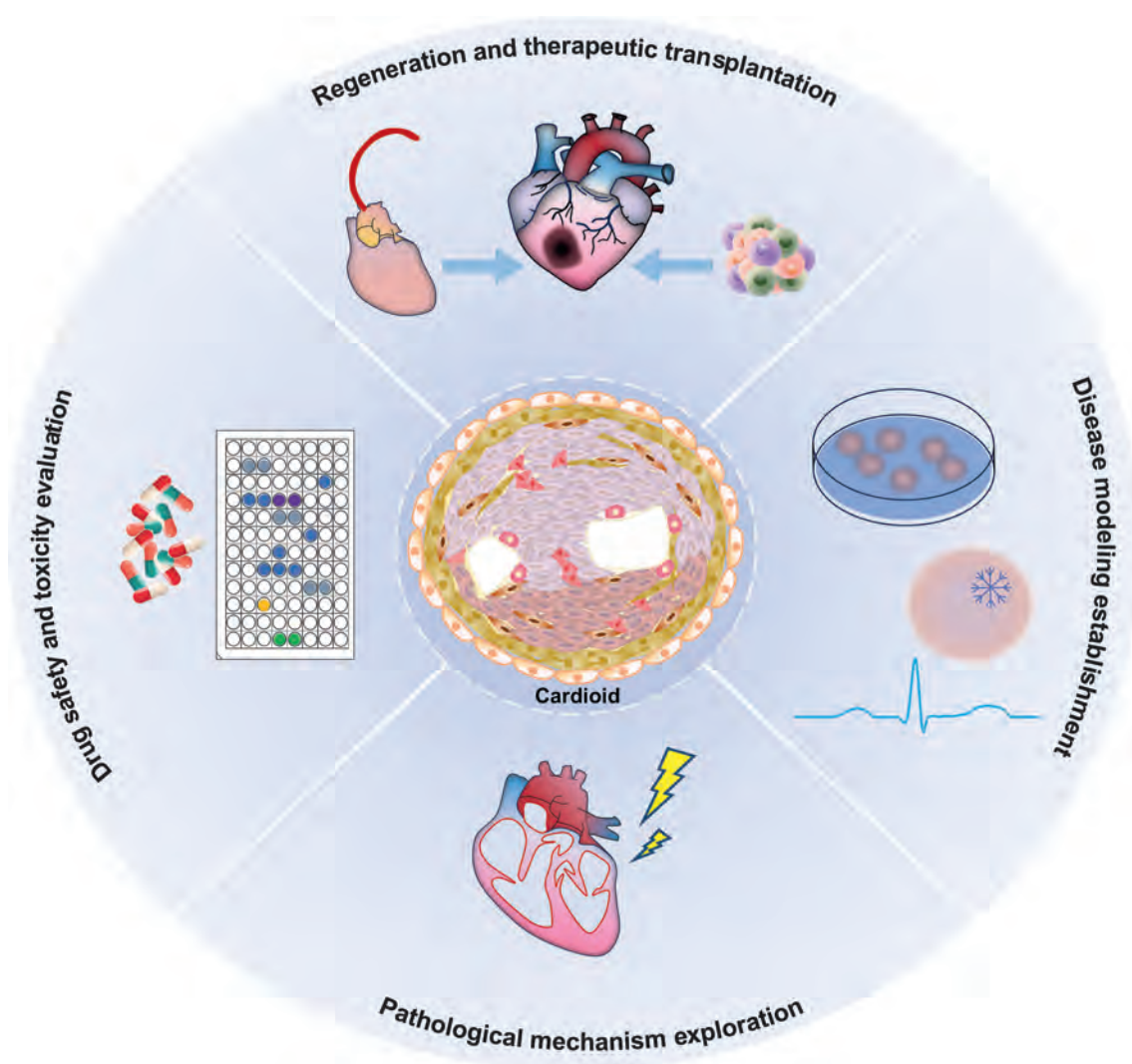


Figure 4 Applications of current human self-organizing cardioid technologies.

Table 3 Disease models established by self-organizing cardioids.

Disease model construction method	Disease modeling	Ref.
High-concentration glucose and insulin stimulation were employed on the basic HOs (three-step WNT stimulation).	PGDHOS: Pregestational diabetes heart organoids for simulating congenital heart defects under specific pregestational diabetes conditions	23
Construct ventricular and atrial heart organoids using the cell line with the <i>NKX2-5</i> gene deleted by the CRISPR/Cas9 strategy.	Genetic lesions at the <i>NKX2-5</i> locus are associated with Ebstein's anomaly	38
<i>NKX2-5</i> knockout heart-forming organoids were conducted by biphasic WNT signal stimulation.	Genetic defects hearts	22
Multi-chamber cardioid (five organoids assembly) by optimizing WNT/BMP signaling.	Investigation of cardiac defects, teratogens, and drug effects	39
hiPSC-derived organotypic cardiac microtissues (multiple cell types transition from 2D culture to 3D multi-cellular heart tissue models).	Chemotherapy-induced cardiotoxicity model	26
Endothelin-1 (ET1) stimulated the epicardioids.	Left ventricular hypertrophy and fibrosis model	29
Cytokine storm [interferon- γ , poly(I:C), and interleukin-1 β] and ET1 stimulated the hVCOs.	Inflammation-induced cardiac dysfunction model	36
Chamber-like cardioids (cardioids with additional epicardium) were contacted with a liquid N ₂ -cooled steel rod.	Cryoinjury model for developmental injury (fibrosis and regeneration)	14
vcCOs contacted by the liquid N ₂ precooled sterile syringe needle.	Cryoinjury model for myocardial injury-induced fibrosis	37
Employs the CRISPR/Cas9 technology to knock out genes, such as <i>ISL1</i> , <i>TBX5</i> , and <i>FOXF1</i> in hPSCs, then constructs different subtypes of cardioids, and artificially assembles these gene-defected cardioids into a multi-chamber cardioid.	Modeling of congenital heart disease (genetic and teratogenic aspects of human cardiac defects)	39

require continuous optimization of technical methods to fully recapitulate *in vivo* cardiogenesis and generate precise miniaturized heart organoids with comparable morphological structures and cellular complexity. This is especially true regarding the mimicry of organoid-like functions such as electrical signal conduction, rhythmic contraction, calcium handling capacity, and cardiac function, as well as in regulating interactions with other tissues or organs.

3. Applications of self-organizing cardioids

Traditional *in vitro* experiments for cardiac research typically rely on primary cardiomyocytes or myocardial cell lines cultured in a two-dimensional environment. While a single pure cell line is convenient for studying the effects of specific conditions on cardiomyocytes, it fails to replicate the intricate cellular interactions and complex structural relationships of the heart within the natural environment. Consequently, experimental outcomes might deviate from real-life scenarios, thereby limiting their significance in disease exploration and guiding drug clinical translation. Self-organizing cardioids are actively addressing these limitations, offering new possibilities for establishing cardiovascular disease models, exploring disease pathogenesis, evaluating drug safety and toxicity, facilitating cardiac regeneration and therapeutic transplantation, as well as other aspects (Fig. 4, Tables 1 and 2).

3.1. Disease modeling establishment and pathogenesis exploration

Current construction methods exhibit varying degrees of similarity to cardiac organogenesis⁵⁸, offering significant advantages for investigating congenital heart defects and pathogenesis. In Table 3^{14,22,23,26,29,36-39}, we summarize current disease model construction methods based on cardioids, roughly including congenital heart defects, genetic disorders, and injury-related conditions (e.g., fibrosis, regeneration). The high-throughput human heart organoids developed by Lewis-Israeli et al.²³ using

a three-step WNT activation-inhibition-activation strategy in 96-well plates exhibit remarkable similarity to human embryonic heart tissue at the transcriptional, structural, and cellular levels. These hCOs could serve as valuable tools for studying congenital heart defects²³. They modified the culture conditions (glucose and insulin levels) to construct pregestational diabetes human heart organoids for mimicking pregestational diabetes-induced congenital heart defects²³. Additionally, Volmert et al.²⁴ further optimized the design scheme and successfully generated gestational cardioids that closely resembled those of 6–10-week-old embryos. These cardioids are more suitable for studying normal heart development, congenital heart defects, cardiac pharmacology, regeneration processes, and other cardiovascular disorders²⁴. As previously introduced, the transcription factor *NKX2-5* plays a crucial role in the early development of the embryonic heart. Studies have further demonstrated that its deficiency is closely associated with Ebstein's Anomaly, a congenital heart defect characterized by the atrialization of the RV and abnormalities of the tricuspid valve⁵⁹. Feng et al.³⁸ established atrial-lineage and ventricular-lineage organoids harboring the *NKX2-5* gene variant linked to Ebstein's Anomaly, thereby demonstrating that this scheme serves as an effective model for investigating atrialized ventricular defects. Similarly, Drakhlis et al.²² found that *NKX2-5* knockout heart-forming organoids (biphasic WNT signal stimulation) exhibited cardiac malformations similar to those observed in *NKX2-5* KO mice, characterized by reduced cardiomyocyte adhesion, hypertrophy, and compaction. Thus, it provides further support for *in vitro* studies on genetic defects in hearts.

The etiology of congenital heart defects extends beyond genetic mutations and encompasses teratogens such as pharmaceuticals, toxins, and metabolic factors⁶⁰. Hofbauer et al.¹⁴ demonstrated the significance of the WNT/BMP4/HAND1 axis in normal cavity morphogenesis using human self-organizing cardioids, providing insights into the molecular mechanisms involved in congenital heart defects development¹⁴. Subsequently, their team once again made contributions in the application of human heart organoids on the studies of teratogenic drugs *via* the

improved scheme of multi-chamber cardioids assembling from five types of organoids³⁹. In this improved scheme, electrophysiological modulators ivabradine and isoproterenol were employed to validate signal propagation between chambers in these multi-chamber cardioids, thus validating the suitability of this platform for discerning early developmental effects of drug and therapeutic agents³⁹. Additionally, the human organotypic cardiac microtissues constructed by Ergir et al.²⁶ were not only applicable to detecting electrophysiological signal propagation but also demonstrated the feasibility of using this model to detect chemotherapy-induced cardiotoxicity by doxorubicin. These are highly informative for studying congenital heart defects and acquired heart diseases caused by drugs, as well as conducting *in vitro* experiments on drug-induced cardiac toxicity. Furthermore, Song et al.²⁵, using a cardiac mesoderm cell-derived COs model, provided novel evidence for the importance of LEFTY–PITX2 signaling activation in heart development, leading to cardiomyocyte maturation and differentiation into ventricular-like cardiomyocyte subtypes.

Meier et al.²⁹ conducted a simulation of left ventricular hypertrophy, a prevalent cardiac condition resulting from both inherited and acquired cardiovascular disorders. They achieved this by administering endothelin-1 (ET1) on their constructed 1-month-old epicardioids, while inducing cardiac fibrosis²⁹. This breakthrough not only successfully established the model of left ventricular hypertrophy and fibrosis, but also provided a novel option for *in vitro* studies on drugs related to congenital and acquired diseases. Voges et al.³⁶ further identified ET1 as a pathological driver of diastolic dysfunction in human vascular cardiac organoids modeling, and they described that it could model both ET1- and cytokine storm (IFN- γ , poly(I:C), and IL-1b)-induced diastolic dysfunction. Additionally, cryoinjury models were separately achieved in chamber-like cardioids¹⁴ and vaschamcardioids³⁷, which could be utilized for developmental injury, simulating the pathological process of myocardial infarction/fibrosis, as well as myocardial regeneration (described in detail below).

3.2. Drug safety and toxicity evaluation

Cardiac toxicity and drug safety are the primary considerations in the clinical use of drugs⁶¹. The self-organizing cardioids, in terms of cellular diversity, spatial structure, and functionality, theoretically overcome the limitations of animal and two-dimensional cultured cell models in accurately describing toxic effects and predicting actual *in vivo* responses. The aforementioned human organotypic cardiac microtissues exhibit significant potential for detecting drug-induced cardiotoxicity, particularly related to chemotherapy drugs²⁶. Temozolomide, a commonly used chemotherapy drug for glioblastoma treatment, has been linked to cardiac toxicity and worsening of heart failure. Using this characteristic, Liang et al.³⁵ employed conditioned medium from tumor cells treated with temozolomide on cardiac organoids to verify whether it would induce similar toxic reactions. The implementation of this procedure broke the integral morphology of cardiac organoids, thereby providing further confirmation that these vascularized cardiac organoids may serve as an improved model for testing cardiotoxic drugs³⁵. Similarly, Yang et al.³⁷ explored the viability of utilizing their constructed multi-lineage human vaschamcardioids as a platform for assessing drug toxicity by employing doxorubicin commonly used chemotherapeutic agent known for its cardiac toxicity. The observed impacts of doxorubicin on beating frequency, contractility, cell viability and apoptosis, and capacity

of calcium handling determination in human vaschamcardioids suggest that the human vaschamcardioids provided a suitable *in vitro* model for evaluating cardiac toxicity of preclinical drugs³⁷. Ondansetron is commonly administered to prevent nausea and vomiting in pregnant women; however, there is limited research on its safety during pregnancy administration⁶². The organoids, built with an enhanced maturation medium 2/1 strategy by Volmert et al.²⁴, recapitulated and revealed potential mechanisms of ondansetron toxicity, including electrophysiological alterations and inhibited ventricular cardiomyocyte differentiation or maturation. Studies indicate that neural crest cells facilitate the maturation of the cardiac conduction system, whereas their removal leads to a delay in this maturation process⁶³. Kostina et al.³³ demonstrated that early exposure to selective serotonin reuptake inhibitors disrupted the normal development of neural crest cell derivatives in human neural crest-heart assembloid (hNCHA). This disruption reduces the expression of critical transcription factors, impairing the formation and function of parasympathetic neurons and glial cells, and ultimately compromising cardiac tissue function³³. hNCHA serves as a valuable model for simulating potential drug-induced risks to cardiac development and provides a robust tool for drug safety assessment. Therefore, these organoids not only serve as models for investigating human heart development and congenital heart defects, but also provide valuable references for the safety assessment of drugs/food during pregnancy. It is widely anticipated that the continuous improvement of cardioid technology will have broader applications in drug/food toxicity testing and safety evaluation.

3.3. Cardiac regeneration and therapeutic transplantation

Human heart organoids hold great potential for application in regenerative medicine as direct substitutes for injured organs or tissues. The cryoinjury model of myocardial infarction in mice is a widely utilized approach to simulate myocardial injury and regeneration⁶⁴. Hofbauer et al.¹⁴ first combined the cryoinjury model with the constructed self-organizing cardioids, resulting in accumulation of ECM, similar to that observed in cardiac regenerative and pathological responses, accompanied by rapid recruitment of endocardial and epicardial fibroblast-like cells.

Furthermore, the research reported by Lee et al.²⁷ demonstrated that chamber-formed human heart organoids with microvascular networks, when transplanted into the subcutaneous tissue of nude mice, still maintained their organoid structure and contraction/relaxation coupling for 10 days. Additionally, these organoids became connected to the surrounding tissue's blood vessels, exhibiting coordinated movement in response to chamber-formed human heart organoid contractions. This provides an important basis for future therapeutic transplantation of cardiac organoids. The epicardial organoid constructed by Wang et al.³⁰ successfully integrates with the adult mouse heart, demonstrating that organoid cells migrate into the inside of the heart. The derived cells exhibited morphological resemblance to native myocardial cells, suggesting that this kind of organoid not only recapitulates key features of the human fetal epicardium but also possesses potential in cardiac regeneration³⁰. Additionally, hematopoietic progenitor cells derived from blood-generating heart-forming organoids, the latest cardioids obtained through a previous heart-forming organoids protocol modification by Dardano et al.³⁴, displayed erythroid, myeloid, and lymphoid multipotency, offering insights into therapeutic transplantation targeting heart hemopoiesis. Currently, research on cardiac regeneration and transplantation using hCOs is

still in its infancy. However, in principle, the specific cells derived from iPSC-induced cardioids can provide a potentially limitless source for transplantation. Additionally, it is worth anticipating advancements in specialized fields of environmental medicine domains, such as aviation medicine.

4. Challenges and prospects

Despite significant advancements in self-organizing heart organoids as described above, there remains a considerable gap to faithfully replicate the chronological sequence of *in vivo* cardiac development and generate accurate miniature cardioids with comparable morphological structures and cellular complexities. These challenges arise from the complex structure and function of the heart, ethical constraints, and the stringent spatiotemporal regulation required for proper cardiac embryonic development⁶⁵. Current cardiac organoids exhibit an immature phenotype resembling fetal hearts and, in certain aspects, are less developed than embryonic hearts. This is mainly due to the mainstream construction methods only relying on signaling pathway (*e.g.*, three-step WNT) stimulation, which causes irregular cardiomyocyte organization and incomplete replication of contraction and relaxation functions. Moreover, the development of the embryonic heart is intricately linked with other germ layers or systems. Although there are multilineage organized cardioids, it is not sufficient, such as the lack of interactions between cardioids and the maternal-like complex environment, as well as the absence of immune and neural cells, further limits the models' ability to fully mimic cardiac functionality. It also shows significant differences in electrophysiological characteristics compared to mature hearts, limiting their ability to accurately replicate heart electrical activities and arrhythmia conditions. As a result, current cardioids may be more suitable for studying cardiogenesis and congenital heart diseases. It is crucial to explore appropriate strategies or integrate other cardiac organoid interventions like scaffold materials or 3D bioprinting for maturing these current organoids into adult-like structures with functional electrophysiology. Additionally, Current techniques of self-organizing cardiac organoids used by different labs are quite different (see details above, Table 2), and there is a lack of unified standards. The results indicate significant variations in the structure, function, and characteristics of heart organoids across different laboratories. Hence, there is an urgent need for a unified framework to assess parameters like size, chamber characteristics, and beating frequency of self-organizing cardioids. It may have great significance in heart disease-related model building, drug screening, cardiogenesis, and pathophysiology research.

From another perspective, inadequate vascularization is another significant problem for cardiac organoids, a challenge that is also frequently observed in other organoids⁶⁶. The lack of vasculature in a cardioid restricts nutrient acquisition to diffusion alone, which is inadequate to sustain healthy growth and survival of internal tissues, thereby hindering the organoid's capacity to maintain normal tissue structure and functionality. The currently existing methods for constructing vascularized cardioids primarily include the ECs co-culture or the vasculogenic growth factors, such as VEGF stimulation, which promote the formation of disordered capillary networks (details in 2.4 section). However, compared to the real cardiac vascular network, it falls short in complexity and functionality. It cannot accurately replicate the branch structure of blood vessels, the hierarchical vascular wall

structure, or their interconnections, and it is also very difficult to achieve true blood perfusion. Common strategies for constructing vascularized organoids may potentially be adapted for cardioids to investigate whether they have potential in developing more mature vascular networks. These strategies might involve tissue engineering techniques using microvascular fragments⁶⁷, scaffold materials⁶⁸, and 3D bioprinting⁶⁹, or transplanting organoids to achieve vascular invasion from the host⁷⁰.

Indeed, the recent advancements in such as 3D bioprinting hearts and engineered heart tissue (EHT) not only highlight significant achievements but also demonstrate great potential for integrated development with the self-organizing cardioids. For example, printing materials recombinant human tropoelastin (a highly biocompatible and elastic bioink) in 3D bioprinting hearts⁶⁹ and ultra-high viscosity alginates for EHT heart patches⁷¹ have demonstrated to bioprint vascularized cardiac constructs and the sensitive electrophysiological response, respectively. These might be attempted to integrated with self-organizing approaches to achieve both vascularization and the fine functions. Combining the EHTs' structural and functional properties might be achieved for providing cardioid tissue-specific microenvironments, electrophysiological properties, or controlling the blood flow direction through using tissue-derived biomaterials, incorporating conductive materials, and fabricating heart valves, respectively⁷². Furthermore, novel scaffold materials might also provide new directions for the improvement of the self-organizing cardioid. Seguret et al.⁷³ developed 3D cardiac rings around a deformable optically transparent hydrogel pillar of known stiffness, and recapitulated inotropic responses to calcium and various drugs (isoproterenol, verapamil). It is mainly through the combination of hiPSCs with adult dermal fibroblasts in an optimized 3:1 ratio that they can self-organize to form ring-shaped cardiac constructs⁷³. To a certain extent, the limitation of the number and structure of cells formed by simple self-tissue was solved. The shrink-resistant collagen-hyaluronan composite hydrogel, recently developed by Oommen's laboratory, is designed to mimic the major components of the extracellular matrix⁷⁴. This hydrogel supports cell encapsulation, exhibits shear-thinning properties, and maintains long-term shape fidelity. It also effectively improves mechanical stiffness, has radical scavenging ability, and provides tissue adhesion⁷⁴. These characteristics could make it promising whether applied in 3D bioprinting or directly for cardiac organoids. It is anticipated that interdisciplinary research in fields such as biomaterials, tissue engineering, optics, and 3D technology, coupled with growing interest from researchers in organoid studies, could address current limitations and yield more refined cardioid models for clinical and scientific research.

5. Conclusions

Recent advancements in construction techniques and applications in self-organizing hCOs have developed rapidly. Broadly speaking, the regulation of identical signals at different time points or distinct signals at the same time points in mesodermal progenitor cells leads to diverse cell types and structures derived from cardioids, which underlie the variations in cardiac composition and features. Moreover, multilineage co-development has also been explored to better emulate the crosstalk between germ layers and multi-organ interactions during cardiogenesis. While the current researches acknowledge certain limitations, it has exhibited considerable potential for diverse applications.

Specifically, cardiac organoids hold promise for mimicking the real heart and provide an excellent platform for studying cardiogenesis mechanisms, conducting drug/food screening, modeling disease, elucidating pathological mechanisms, and investigating specialized environmental medicine.

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Author contributions

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Conflicts of interest

The authors declare no conflicts of interest.

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