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Revolutionizing cardiovascular research: Human organoids as a Beacon of hope for understanding and treating cardiovascular diseases

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ABSTRACT

Organoids, exhibiting the capability to undergo differentiation in specific in vitro growth environments, have garnered significant attention in recent years due to their capacity to recapitulate human organs with resemblant in vivo structures and physiological functions. This groundbreaking technology offers a unique opportunity to study human diseases and address the limitations of traditional animal models. Cardiovascular diseases (CVDs), a leading cause of mortality worldwide, have spurred an increasing number of researchers to explore the great potential of human cardiovascular organoids for cardiovascular research. This review initiates by elaborating on the development and manufacture of human cardiovascular organoids, including cardiac organoids and blood vessel organoids. Next, we provide a comprehensive overview of their applications in modeling various cardiovascular disorders. Furthermore, we shed light on the prospects of cardiovascular organoids in CVDs therapy, and unfold an in-depth discussion of the current challenges of human cardiovascular organoids in the development and application for understanding and treating CVDs.

1. Introduction

Cardiovascular diseases (CVDs) stand as the leading causes of mortality and disability worldwide, representing a significant contributor to the global public health burden [1]. Despite ongoing efforts by scientists to fathom the intricate pathophysiological mechanisms of CVDs with the aim of developing innovative drugs, the incidence of CVDs continues to escalate annually. To date, the landscape of drug development predominantly relies on conventional animal models. Prior to embarking on the inaugural human clinical trials, the safety and efficacy of drugs undergo rigorous testing in animal subjects. However, substantial disparities exist between animals and humans in terms of physiological and pathological environments, significantly compromising the outcomes and advancement of clinical trials of CVDs drugs.

With the increasing discrepancies in key findings between traditional animal models and human clinical applications, scientists are actively seeking innovative biological models to address this challenge [2]. Classic cell cultures and traditional animal models have been widely employed in drug development and preclinical research. These models are renowned for their robustness, rapid growth, and high reproducibility, rendering them cost-effective and easily managed in laboratory settings [3]. To date, the mouse model remains the most commonly used organism for studying human diseases, including CVDs [4]. The adoption of the mouse model for studying human diseases gained traction in the early 20th century. Mice offer advantages such as faster breeding, reduced floor area requirements, and ease of housing and care, making them a practical choice for experimental studies [5]. However, animals are unable to precisely replicate human biological processes and physiological conditions due to their significant differences in genetic background, physiology, and anatomy. For instance, the proportion of white matter in rodent brains is notably lower compared to that in the human brain. While human brains have approximately 60 % white matter, rodents have a proportion not exceeding 15 % [6]. The left atrium of the human heart receives blood from four distinct pulmonary

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veins, while in mice, the pulmonary veins converge into the left atrium through a single opening [7]. In addition, the human heart is significantly larger and more complex than the murine heart. Mice exhibit higher heart rates, and this disparity in cardiac function can influence disease pathogenesis, particularly in conditions such as arrhythmias and heart failure [7]. Recently, the fabrication of human organoids has gained widespread attention as a potential breakthrough in addressing these challenges, offering a pathway to the development of more effective and clinically relevant drugs for CVDs therapy [8,9].

Organoids refer to cells flourishing within in a controlled three-dimensional (3D) setting in vitro, leading to small groups of cells that autonomously organize and differentiate into functional organ-like 3D miniature, which can recapitulate the key structures and functions of native organ in vivo [10]. Organoids can be generated from human pluripotent stem cells (hPSCs) and adult stem cells (adSCs) through two prevalent methods including self-assembly and direct assembly [11,12], and both cell types are compatible with most standard techniques of organoid fabrication [13–15]. Cardiovascular organoids (CVOs), including cardiac organoids (COs) and blood vessel organoids (BVOs), have substantially advanced the development of in vitro 3D non-animal models of various CVDs such as myocardial infarction (MI), heart failure, diabetes-related vascular complications, cardiomyopathy and arrhythmia [16]. Moreover, human CVOs have shown great potential in CVDs drugs development, toxicity testing, personalized medicine, regenerative medicine, and transplantation therapy [8,17,18]. In this review, we outline the development of human cardiovascular organoid culture and provide an overview of the key techniques for creating CVOs. We highlight their applications and prospects in cardiovascular research. Additionally, we discuss the potential challenges and limitations that must be addressed to further advance and expand the use of CVOs in CVD research.

2. Development and manufacture of human cardiovascular organoids

2.1. The development of cardiovascular organoids

Conventional cardiovascular cell models have predominantly relied on two-dimensional (2D) culture systems, which are inherently limited in their ability to accurately recapitulate native cardiovascular tissues development and disease. These limitations arise from the inability of 2D cultures to replicate the morphogenetic and pathophysiological processes that depend on critical cell-cell and cell-extracellular matrix (ECM) interactions, which are essential for understanding CVDs [19,20]. To address the limitations of traditional 2D cultures, in vitro 3D culture models that more accurately mimic the complex structures and functions of the native heart and blood vessels have been developed [21,22]. The concept of 3D cell culture, a precursor to organoid technology, emerged from the observation that cells behave differently when cultured in a 3D environment compared to traditional 2D monolayers. In the 1970s, researchers began exploring cell culture in 3D matrices, such as collagen and Matrigel, which offered a more in vivo-like microenvironment that supported cellular growth and differentiation (Fig. 1) [23]. Furthermore, the emergence of pluripotent stem cells (PSCs), with their remarkable capacity to differentiate into various types of cardiovascular cells such as endothelial cells (ECs), smooth muscle cells and

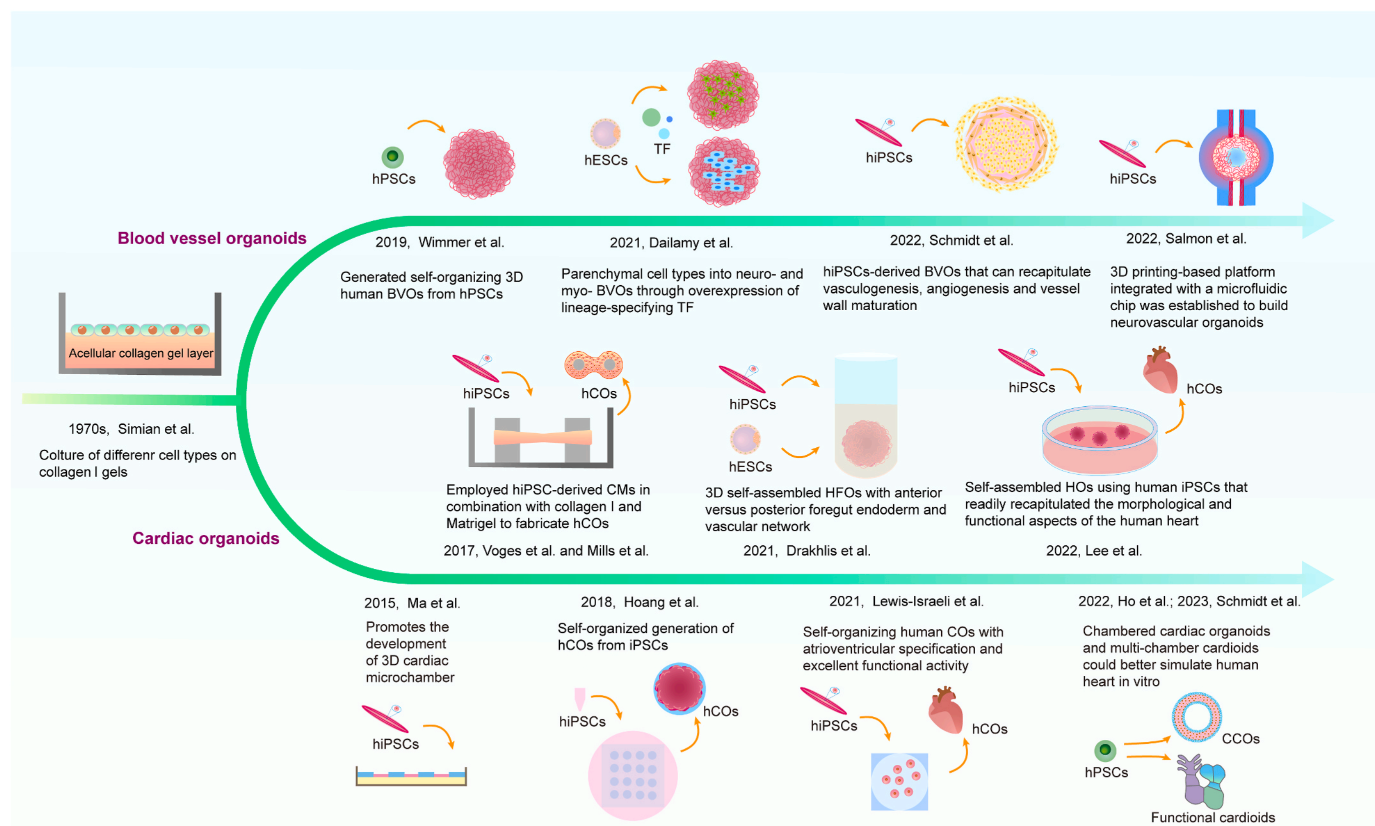

Fig. 1. The developmental trajectory of human cardiovascular organoids. Cardiovascular organoids can be classified into blood vessel organoids (BVOs) and cardiac organoids (COs). In 2019, functional 3D vascular organoids were introduced, primarily used for vascularizing other tissue structures to simulate the microenvironment of human blood vessels. The development of 3D cardiac organoids can be traced back to 2015. The advent of 3D cardiac microchambers significantly promoted the development of functional 3D cardiac organoids, with self-organized cardiac organoids appearing in 2018. In 2021, cardiac organoids with chamber function emerged, more closely resembling the real human heart. hPSCs: human pluripotent stem cells, hiPSCs: human induced pluripotent stem cells, hESCs: human embryonic stem cells, HFOs: heart-forming organoids, TF: transcription factor.

cardiomyocytes (CMs), has enabled researchers to generate in vivo-like CVOs, including blood vessel organoids and cardiac organoids, that more accurately recapitulate the structure and function of human cardiovascular tissues [9,24].

2.1.1. Blood vessel organoids

Blood vessel organoids (BVOs), mimicking the structural and functional characteristics of blood vessels, can be derived from hPSCs that can differentiate into various types of vascular cells, including ECs, pericytes, and smooth muscle cells [25]. In 2013, the self-assembly of hPSCs into vascular structures within a synthetic matrix was established to lay the groundwork for the development of 3D vascular organoids (Fig. 1) [26]. In 2019, Wimmer et al. successfully generated self-organizing 3D human BVOs from hPSCs through mesoderm induction of hPSC aggregates followed by differentiation into endothelial networks and pericytes within a 3D collagen I-Matrigel matrix, exhibiting morphological, functional, and molecular characteristics of human microvasculature (Fig. 1) [27,28]. Importantly, when transplanted into mice, these human BVOs can form a stable, perfused vascular tree, including arteries, arterioles and venules [27,28]. Next, in 2021, Dailamy et al. proposed a programmatic introduction of parenchymal cell types to create neuro- and myo-BVOs that maintain neural and vascular function for at least 45 days in culture through overexpression of lineage-specifying transcription factor (TF) (Fig. 1) [29]. In 2022, Schmidt et al. introduced a novel and cost-effective protocol to generate more sophisticated BVOs that can recapitulate human vasculogenesis, angiogenesis and vessel wall maturation by seeding human induced pluripotent stem cells (hiPSCs) in non-adhesive agarose-coated wells and inducing them towards a lateral plate mesoderm fate through activation of Wnt and bone morphogenetic protein 4 (BMP4) signaling, providing a platform to study all stages of blood vessel development and maturation (Fig. 1) [30]. Furthermore, a 3D printing-based platform integrated with a microfluidic chip was established to build neurovascular organoids through a synchronized co-culture of hiPSC-derived pericytes and ECs (Fig. 1) [31].

2.1.2. Cardiac organoids

Cardiac organoids (COs), resembling human heart tissues, can be derived from hPSCs that can differentiate into various types of cardiac cells [9,24], and offer higher physiological relevance, organ-specific functions, and patient-specific features [9,24]. The development of early COs began in 2015 when Ma et al. reported that the establishment of a beating human 3D cardiac microchamber, confined by the pattern geometry, through the synergistic differentiation of patterned hiPSCs into CMs (Fig. 1) [32]. In 2017, Voges et al. and Mills et al. employed hiPSC-derived CMs in combination with collagen I and Matrigel to fabricate human cardiac organoids (hCOs), which displayed characteristics of immature human myocardium and exhibited physiological inotropic responses to calcium (Fig. 1) [33,34]. To experimentally model early cardiac organogenesis in vitro, in 2018, researchers created 3D spatially patterned early-developing COs with distinct organization and self-assembly by combining biomaterial-based cell patterning with stem cell organoid engineering (Fig. 1) [35]. Subsequently, in 2021, complex, highly structured and 3D self-assembling heart-formation organoids with a myocardial layer lined by endocardial-like cells distinct, anterior versus posterior foregut endoderm and vascular network was fabricated by embedding human pluripotent stem cell aggregates in Matrigel and subsequently directing cardiac differentiation through biphasic Wnt pathway modulation (Fig. 1) [36]. In the same year, Lewis-Israeli et al. reported that self-organizing human COs with well-organized multi-lineage cardiac cell types, atrioventricular specification, and excellent functional activity were generated based on hPSC through a three-step Wnt signaling modulation strategy (Fig. 1) [8]. Furthermore, in 2022, Lee et al. developed more mature self-assembled heart organoids (HOs) using human iPSCs that exhibited validated mimicry of cardiac structures such as the chamber, epicardium/myocardium, atrium/ventricle-similar areas, vascularization after in vivo transplantation, as well as mechanical/-electrophysiological features, which can closely and readily recapitulate the morphological and functional aspects of the human heart (Fig. 1) [37]. In the same year, researchers successfully generated matured human chambered cardiac organoids (CCOs), which could be used to model clinical features of cardiac hypertrophy, exhibiting thickened chamber walls, reduced fractional shortening, and increased myofibrillar disarray upon treatment with endothelin-1, from hESCs (H7) through modulation of Wnt/β-catenin signaling and growth factors [38]. To study embryogenesis and the development of embryonic heart, multi-chamber cardioids that recapitulate the development of all major embryonic heart compartments, including right and left ventricles, atria, outflow tract, and atrioventricular canal, were developed by Schmidt et al., in 2023, using progenitors of the first heart field, the second heart field, and the posterior second heart field [39].

2.2. Manufacture of human cardiovascular organoids

In general, there are two prevalent methods employed for human CVOs production, including self-assembly (or self-organization) [8,36,40–42] and direct assembly [43–45] (Table 1). In the approach of self-assembly, cells, typically derived from PSCs, are allowed to spontaneously aggregate and organize into organoid structures. Directed assembly techniques can promote the formation of aggregates, which typically refer to clusters of cells assembled under specific conditions to promote cell-cell interactions and self-organization. These clusters can be directed to develop into more complex, functional structures with features of specific organs, which we refer to as organoids. Direct assembly involves a more controlled approach, where cells or tissue components are assembled in a predetermined manner, often with the help of bioengineering techniques such as 3D bioprinting or microfabrication, allowing for greater control over the spatial organization and architecture of the generated organoids (Fig. 2). Currently, several techniques including suspension culture, hydrogel/matrix embedding, 3D bioprinting, and microfluidics, are commonly employed for the production of human CVOs.

2.2.1. Suspension culture

In the suspension culture system, cells are grown in a floating state rather than being attached to a surface. The cells are typically placed in spinner flasks or bioreactors, where continuous stirring facilitates the growth of 3D organoids without the need for scaffolds. Suspension culture is a well-established method for generating COs. Lewis-Israeli et al. employed suspension culture to generate self-assembling human heart organoids by seeding 10,000 hPSCs (iPSC-L1, AICS-0037-172, iPSCORE_16_3, iPSC GCaMP6f, and human ESC line H9) in 96-well plates and sequentially exposing them to CHIR99021, B27, and Wnt-C59 to activate, inhibit, and then reactivate the Wnt pathway for differentiation into hHOs (Fig. 2A) [8,46]. Recently, an efficient and reproducible cardiac differentiation protocol has been developed to produce COs by utilizing stirred suspension systems and cost-effective spinner flasks. In this culture system, WTC-11, a wild-type human male iPSC line, was allowed to be differentiated into cardiomyocytes (hiPSC-CMs) through a 2D monolayer culture treated with Y-27632, CHIR99021, and IWR-1-endo. After 8 days of differentiation, the culture was transferred to a magnetic stirring bottle, and bioreactor-derived COs were formed after 15 days of suspension culture (Fig. 2A) [47]. This approach eliminates direct substrate contact, facilitating the self-assembly of cardiac tissue, and represents the first cardiac organoids entirely formed in a bioreactor. Suspension culture is not widely employed for the production of BVOs. Nonetheless, self-organizing 3D human BVOs generated from hPSCs within a 3D collagen I-Matrigel matrix can be further cultured in scalable suspension culture [28]. Hence, suspension culture supports high-throughput production of organoids with fewer limitations from substrate interaction.

Table 1
Manufactures and applications of various cardiovascular organoids (CVOs).

Types of CVOs	Cell sources	Manufacture methods of CVOs	Cell types in CVOs	Application in CVDs modeling	Reference
Cardiac organoids	hPSCs	Self-assembly	Cardiomyocyte, epicardial cells, fibroblasts, endocardial cells, and endothelium	Recreate complex metabolic disorders associated with congenital heart defects	[8]
	hESCs and hiPSCs		Cardiomyocyte and endocardial cells	Model resembles aspects of the decreased cardiomyocyte adhesion and hypertrophy resulting from NKX2.5 loss in native heart development	[36]
	hPSCs		Cardiomyocyte, epicardial cells, fibroblasts, endocardial cells, and endothelium	Study mechanisms of human cardiogenesis and heart disease	[40]
	mESCs		Cardiac progenitors	Organoid model that mimics the early development of the heart	[42]
	hiPSCs		Cardiomyocytes, fibroblasts, and endothelial cells	Mimicking acute myocardial infarction and cardiac fibrosis using HOs	[136]
	hPSCs		Cardiomyocytes and epicardial cells	Pregestational diabetes induced congenital heart defects	[67]
	hiPSCs		Cardiomyocytes, endothelial cell, sinoatrial node-related cells, epicardial cells, proepicardial cells, cardiac fibroblasts and mesenchymal cardiac progenitors	This model enables in vitro observation of the molecular mechanisms underlying diabetic embryonic heart disease.	[71]
	hiPSCs		Atrial cardiomyocytes, ventricular cardiomyocytes, proepicardial-derived cells, epicardial cells, endothelial cells, and valve cells	Exploring the effects of ondansetron that a drug administered to pregnant women and associated with congenital heart defects	[41]
	hiPSCs		Cardiomyocyte, endothelial cells and fibroblast/adipose progenitors	Gain insight into the disease mechanism of DMD-associated hypertrophic/dilated cardiomyopathy,	[94]
	hiPSCs		Myocardial and endocardial	Cardiac organoid was the myocarditis valid infection model in vitro	[70]
	hiPSCs		Cardiomyocytes	Establish short QT syndrome model and assessing drug treatments	[68]
	hiPSCs		Cardiomyocytes, vascular endothelial cells, fibroblasts, and smooth Muscle Cells	Various drugs can be tested for their effects on electrical activity in arrhythmia modeling	[99]
	hiPSCs		Cardiomyocytes and smooth muscle-like stromal cells	As an in vitro platform for studying organoid structure-function relationships, developmental processes, and drug-induced cardiac developmental toxicity	[103]
	hESCs		Cardiac progenitor cells, mesenchymal stem cells and endothelial cells	By transplantation of COs into the peritoneal cavity of nude mice induced neovascularization in CO, resulted in more ultrastructural organization of myofibrils	[111]
	hPSCs		Cardiomyocytes and cardiovascular progenitor cells	MSC-derived extracellular vesicles may serve as a potential therapeutic approach for preventing or mitigating damage caused by ischemia-reperfusioninjury in cardiac tissues.	[137]
	hiPSCs		Cardiomyocytes, fibroblasts, and endothelial cells	The combination of hiPSC and organoid technology makes the production of human 3D cardiac organoid closer to physiology.	[138]
Cardiac aggregates	hiPSCs	Directed assembly	Cardiomyocytes, myofibroblasts and smooth muscle cell	Drug cardiac developmental toxicity screening	[32]
	hPSCs		Cardiomyocytes, human cardiac fibroblasts, endothelial cells, and stromal cells	Conductive silicon nanowires enhance the therapeutic efficacy of engineered human cardiac microtissues for heart repair	[114]
	hiPSCs		Cardiomyocyte and fibroblasts	Cardiac maturation/adult disease modeling	[45]
	hPSCs		Cardiomyocyte, epicardial cells, fibroblasts, pericyte, endothelial cells and myocardial cells	Developing drugs to prevent heart infections and damage	[44]
	hiPSCs and hESCs		Cardiomyocytes, myofibroblast/smooth muscle cell	Embryotoxicity screening/structural malformations at early stage of cardiac organogenesis	[35]
	hiPSCs		Fibroblast and cardiomyocyte	Organoids as myocardial infarction model can model hypoxia-enhanced doxorubicin cardiotoxicity	[66]
	hPSCs		Cardiomyocyte and fibroblast	In vitro simulation of heart failure, drug evaluation and development of heart repair patch	[69]
	hiPSCs		hiPSC- cardiomyocytes, human cardiac microvascular endothelial cells, and human cardiac fibroblasts	Organoids are capable of representing significant phenotypical features of the healthy and hypertrophic cardiomyopathic human heart in vitro	[93]
	hiPSCs		Fibroblasts. cardiac myocytes and endothelial cells	Drug screening	[102]
	hPSCs		Cardiomyocytes, fibroblasts, endothelial cells and smooth muscle cell	hCO platforms can be used in drug discovery pipelines to identify the most efficacious activators of cardiomyocyte proliferation	[101]
Blood vessel organoids	hPSCs	Self-assembly	VE-Cadherin + endothelium, mesenchymal stem-like cells, haematopoietic cells and pericytes	Establish diabetic microvascular pathologies model and drug screening and evaluation	[27]
	hESC (H9) and hiPSCs		Endothelial cells and pericyte	Investigate diabetic vascular basement membrane thickening	[28]
	iPSCs		Endothelial cells and pericytes	As a model to study the mechanisms of microangiopathy	[48]
	hiPSCs, human fibroblasts and endothelial cells		Human umbilical vein endothelial cells, fibroblasts, endothelial cells and pericytes	Improved growth and functionality of 3D tissues via vascularization	[60]

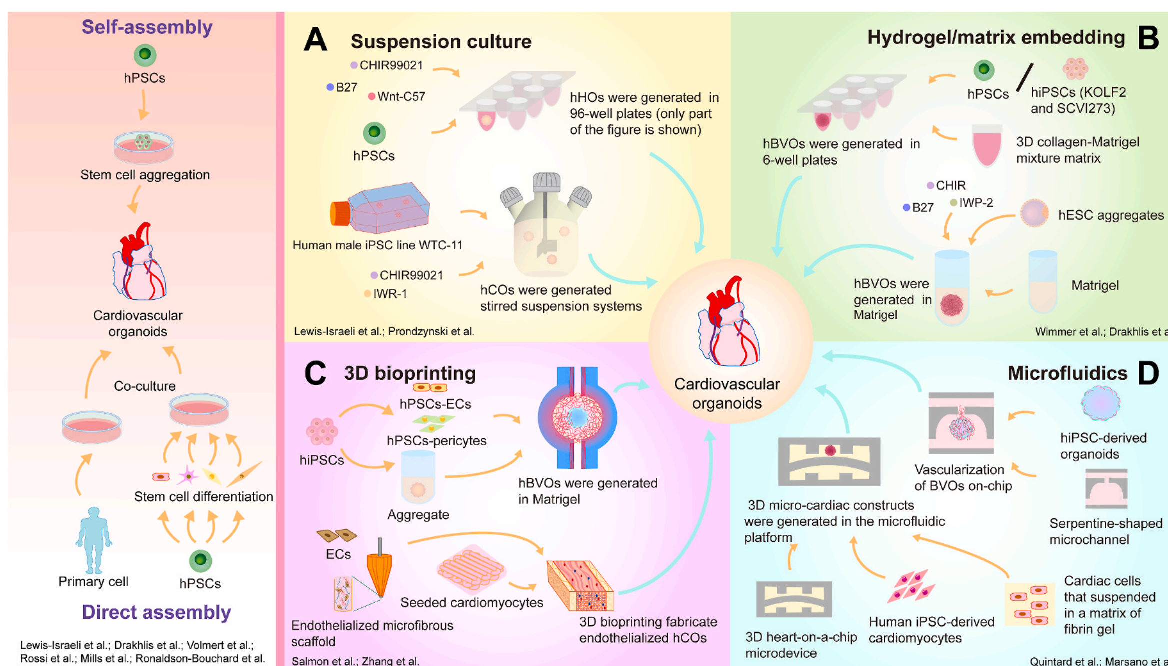

Fig. 2. The manufacturing techniques of human cardiovascular organoids. Human CVOs are primarily produced through two methods: self-assembly (or self-organization) and direct assembly. Currently, there are four techniques for fabricating human cardiovascular organoids: (A). Suspension culture; (B). Hydrogel/matrix embedding; (C). 3D bioprinting; (D). Microfluidics.

2.2.2. *Hydrogel/matrix embedding*

Cells are embedded in natural or synthetic hydrogels, such as collagen or Matrigel, which provide a supportive scaffold mimicking the extracellular matrix, allowing cells to form 3D structures within a biologically relevant environment. Within a 3D collagen-Matrigel mixture matrix, self-organizing 3D human BVOs exhibiting morphological, functional, and molecular features of microvasculature and vascular networks were successfully generated within 2–3 weeks through mesoderm induction of hPSC or hiPSCs (KOLF2 and SCVI273) aggregates, followed by differentiation into endothelial networks and pericytes (Fig. 2B) [28,48,49]. hCO with immature human myocardium can be constructed using differentiated cardiac cells embedded in collagen I for 5 days in the mold [33]. Moreover, hPSC-derived cardiomyocytes can also serve as a cell source for generating hCOs through the hydrogel/matrix embedding technique [34]. Complex and highly structured 3D heart-forming organoids is able to be generated by embedding hESC lines (HES3 NKX2.5–eGFP, HSC_ADCF_SeV-iPS2, HES3 MIXL1–GFP, and HES3 NKX2.5–eGFP/eGFP) as cell aggregates in Matrigel, followed by directed cardiac differentiation using biphasic WNT pathway modulation with small molecules (Fig. 2B) [36]. Through the treatment with B-27, CHIR, and IWP2, the hPSC aggregates underwent progressive differentiation, resulting in organoids approximately 2 mm in diameter within 14 days [36].

2.2.3. *3D bioprinting*

3D bioprinting uses bio-inks composed of living cells and biomaterials to create complex, layered structures with high spatial precision, which enables the creation of geometrically defined 3D structures that significantly enhance their physiological relevance through architectural mimicry of native tissues and organs [50,51]. 3D tissue constructs containing vasculature, human neonatal dermal fibroblast cells, primary human umbilical vein endothelial cells (HUVECs), and extracellular matrix have been produced through the precise co-printing of bioinks in 3D [52]. In 2019, by using cells and biological material from a human patient bioprinting, Israeli researchers successfully bioprinted the first synthetic 3D vascularized engineered heart, encompassing blood arteries, ventricles, and chambers [53]. To investigate the effects of microgravity on CVDs, Alonzo et al. fabricated a 3D bioprinted hCOs characterized by sustained longevity and function involved printing annular ring-like scaffolds composed of a hydrogel (gelatin-alginate) construct encapsulated with a mixture of human cardiac AC16 CMs, fibroblasts (FBs), and microvascular ECs [54]. A 3D printing-based platform integrated with a microfluidic chip can facilitate a sequential and developmentally synchronized co-culture of human pluripotent stem cell-derived pericytes and ECs, which enables the sprouting and self-assembly of these cells into organized vascular networks, promoting the formation of neurovascular organoids (Fig. 2C) [31]. A novel hybrid technique based on 3D bioprinting by utilizing composite bioink, hiPSC-CMs, ECs directly bioprinted within microfibrous hydrogel scaffolds has been developed to fabricate endothelialized COs and heart tissues exhibiting spontaneous and synchronous contraction (Fig. 2C) [55]. Furthermore, a 3D hCOs, featuring distinct geometries and blood vessel-like structures, was successfully generated using a multi-cellular 3D bioprinting approach that incorporated heterogeneous, multi-cellular constructs composed of HUVECs and iPSC-CMs [56].

2.2.4. *Microfluidics*

Microfluidics refers to a system that controls fluid flow using microchannels at the submillimetre scale to recreate the dynamic microenvironment [57], which can support vascularization and enable real-time observation of CVOs development and function [58]. This system allows to simulate the key physiological conditions of cardiovascular system, such as vascular network formation, electrical stimuli, shear stress, and mechanical stretch. A microfluidic chip seeded with mesenchymal stem cells, SMCs, or HUVECs was developed to simulate the hemodynamic microenvironment of blood vessels in vivo by simultaneous or individual fluid shear stress and cyclic stretch to vascular cells, which demonstrated reliability in replicating the hemodynamic environment and structure and function of blood vessels [58]. A microfluidic system was developed to model blood vessel formation and angiogenesis by incorporating various stages of vascular development, including vasculogenesis, endothelial cell lining, sprouting angiogenesis, and anastomosis [59]. This system utilized human endothelial colony-forming cell-derived endothelial cells and human lung fibroblasts to generate and

maintain a 3D microvascular network, providing a physiological transport model of interconnected, perfused vessels, from arteries to vascularized tissue and veins [59]. Additionally, a microfluidic chip platform integrated with 3D bioprinting was developed to fabricate neurovascular organoids by inducing the organization of human pluripotent stem cell-derived pericytes and ECs into vascular networks through sprouting and self-assembly [31]. Recently, a novel technique integrating microfluidic chips and functional BVOs-on-chip was established to monitor the formation of endothelial networks surrounding mesenchymal and pancreatic islet spheroids, as well as BVOs derived from hiPSC line NC8, cultured on-chip for up to 30 days [60]. This microfluidic platform is the first device to incorporate functional vasculature throughout microfluidic-trapped, embedded 3D blood vessel organoids (BVOs), enabling the generation of perfused hierarchical networks that include vessels ranging in size from arterioles to venules and capillaries (Fig. 2D) [60]. The microfluidic system can be also used to facilitate producing hCOs. A microfluidic patterning technique utilizing hyaluronic acid (HA) substrates as inductive templates was employed to fabricate 3D hCOs [61]. Upon seeding, CMs elongate and align along the patterned direction, attaching primarily to the glass substrate and the interface between the HA patterns and glass [61]. After three days in culture, the linearly aligned myocytes detached from the surface, forming contractile COs [61]. This method offers a simple, rapid, and cost-effective approach for creating in vitro hCOs. In addition, Marsano et al. constructed a novel microfluidic system to generate mature and highly functional micro-engineered hCOs that can highly recapitulate the physiologic mechanical environment in the native myocardium from both neonatal rat and hiPSC-CMs [62]. This system, featuring an array of hanging posts to constrain cell-laden gels and a pneumatic actuation system promoting homogeneous uniaxial cyclic strains to the 3D cell constructs, offers a standardized 3D cardiac model for predicting hypertrophic changes in cardiac phenotype through mechanical and biochemical co-stimulation (Fig. 2D) [62]. For the fast-paced high-content imaging and visualization of COs, a specialized microfluidic imaging system was developed by immobilizing COs in predetermined locations for viability staining and calcium-transients imaging without altering their morphology and function [63]. This system includes six parallel trapping areas, each equipped with a staging and immobilization chamber, designed to receive organoids transferred from their native culture plates [63].

2.3. Functional evaluations of cardiovascular organoids

To ensure the successful generation of CVOs, it is essential to evaluate their key functional properties that mimic the native cardiovascular systems. For BVOs, assessments should include vascular tube and network formation, endothelial cell function, angiogenic potential, immune responses, extracellular matrix remodeling, and vasoconstriction/vasodilation [30,64,65]. For COs, it is crucial to examine key functional attributes that reflect their physiological relevance and maturity of heart, including contractile behavior, force generation, rhythm, wall thickness, and ejection fraction [8,17,38]. Evaluating the functions of blood vessel organoids and cardiac organoids requires a range of analytical methods, including microscopic imaging techniques, angiogenesis and network formation assays, electrophysiology, contractility assay, calcium imaging assay and molecular analysis, to assess structural integrity, physiological function, molecular expression, and response to stimuli. Confocal and fluorescence microscopy can be used to visualize structural markers, cell types, and proteins within the cardiovascular organoids [66,67]. Confocal imaging allows for high-resolution, three-dimensional reconstructions, essential for examining the spatial organization of vascular networks and cardiac muscle layers [27]. Live-cell imaging enables real-time monitoring of cellular dynamics, such as contractile behavior in COs and the formation and remodeling of blood vessels in vascular organoids [27,32,39]. Electron microscopy offers ultrastructural insights into organoids, including sarcomere organization in COs and the integrity of endothelial junctions in blood vessel organoids [27]. Angiogenesis and network formation assays are essential to evaluate the formation of new vessels, including measurements of branch points, vessel length, and network density [27]. Video-based contractility measurements or traction force microscopy assess the mechanical beating properties of cardiac organoids, providing information on force generation and rhythmicity, which can be used to study heart muscle function or drug effects on cardiac contractility [68–70]. qRT-PCR and RNA sequencing can be used to analyze the expression levels of key genes associated with vascular or cardiac function, such as genes involved in angiogenesis, cardiac contractility, ion channels, and immune response, which are essential for identifying molecular changes in response to drugs or genetic modifications [27,69–71]. Immunostaining enables detection of nitric oxide (NO) production, and cell-type-specific markers, such as CD31 for endothelial cells in vascular organoids and troponin for cardiomyocytes in COs, to confirm the presence and spatial distribution of desired cell types within the organoid structure [27,66,67,70]. Patch-clamp or multi-electrode array (MEA) techniques measure electrical activity in COs, providing data on action potentials, conduction velocity, and arrhythmic activity, which are critical for modeling cardiac arrhythmias or testing drug responses [68]. Calcium imaging with fluorescent calcium indicators (e.g., Fluo-4) is frequently used to monitor intracellular calcium transients in COs, which are indicators of electrical activity and excitation-contraction coupling [66,71]. The genetically encoded calcium indicator for optical imaging (GCaMP), a widely used tool for measuring calcium dynamics in live cells, tissues, and organoids, enables real-time visualization of calcium transients, a crucial marker of cellular activity, particularly in excitable cells like cardiomyocytes [66,72]. GCaMP offer high-resolution, spatiotemporal data on calcium transients, which are key indicators of electrophysiological activity and contractility [73]. This technique allows visualization of calcium wave propagation, rhythm synchronization, and functional maturation across the organoid [73]. It is particularly well-suited for large-scale, high-throughput applications due to its capacity to simultaneously monitor activity in multiple regions of an organoid without direct contact. In contrast, MEA provide a robust, non-invasive platform to measure extracellular electrical activity [74,75]. MEAs offer excellent temporal resolution and can record action potential propagation and electrical coupling across the organoid, making them ideal for assessing arrhythmogenic potential and long-term electrical stability [68]. While their throughput is lower than calcium imaging, MEAs provide more direct insights into electrophysiological properties without requiring fluorescent reporters. In addition, patch clamping is the gold standard for measuring ionic currents and action potentials at the single-cell level [76]. This technique provides unparalleled accuracy in characterizing ion channel activity and cellular excitability but is labor-intensive, requires specialized expertise, and has very low throughput compared to the other methods [77]. Nevertheless, each of these analytical methods provides unique insights into the functionality, structural integrity, and response of blood vessel and cardiac organoids, allowing researchers to comprehensively assess organoid performance and relevance as disease models. Nonetheless, combining these methods can improve the characterization of organoids, making them more predictive and robust models for disease research and drug development.

2.4. Automation and artificial intelligence in cardiovascular organoids culture and analysis

The integration of automation and artificial intelligence (AI) in cardiovascular organoid culture and data analysis is revolutionizing cardiovascular research. Automation is being used to scale up the production and maintenance of cardiac organoids, enabling high-throughput screening for drug discovery and toxicity testing [78,79]. A high-throughput automated method for the suspension culture and real-time analysis of individual gastrointestinal organoids within a

polymer-hydrogel substrate has been developed via stem-cell aggregation in microcavity arrays, which eliminates the need for a solid matrix and significantly reduces organoid heterogeneity [80]. This approach allows for the efficient handling of large numbers of organoids, ensuring consistency and reproducibility. Moreover, standardized and automated protocols for the generation and culture of cardiac organoids help in reducing variability and improving the reliability of experimental outcomes [81]. Dynamic changes in individual organoid morphology, number, and size can indicate important drug responses. Artificial intelligence (AI) platforms like OrganoID are being employed to track and analyze single-organoid dynamics. These platforms use deep learning to automatically recognize, label, and track organoids in microscopy images, providing detailed morphological and functional data [82]. AI is also used to create predictive models that can simulate disease

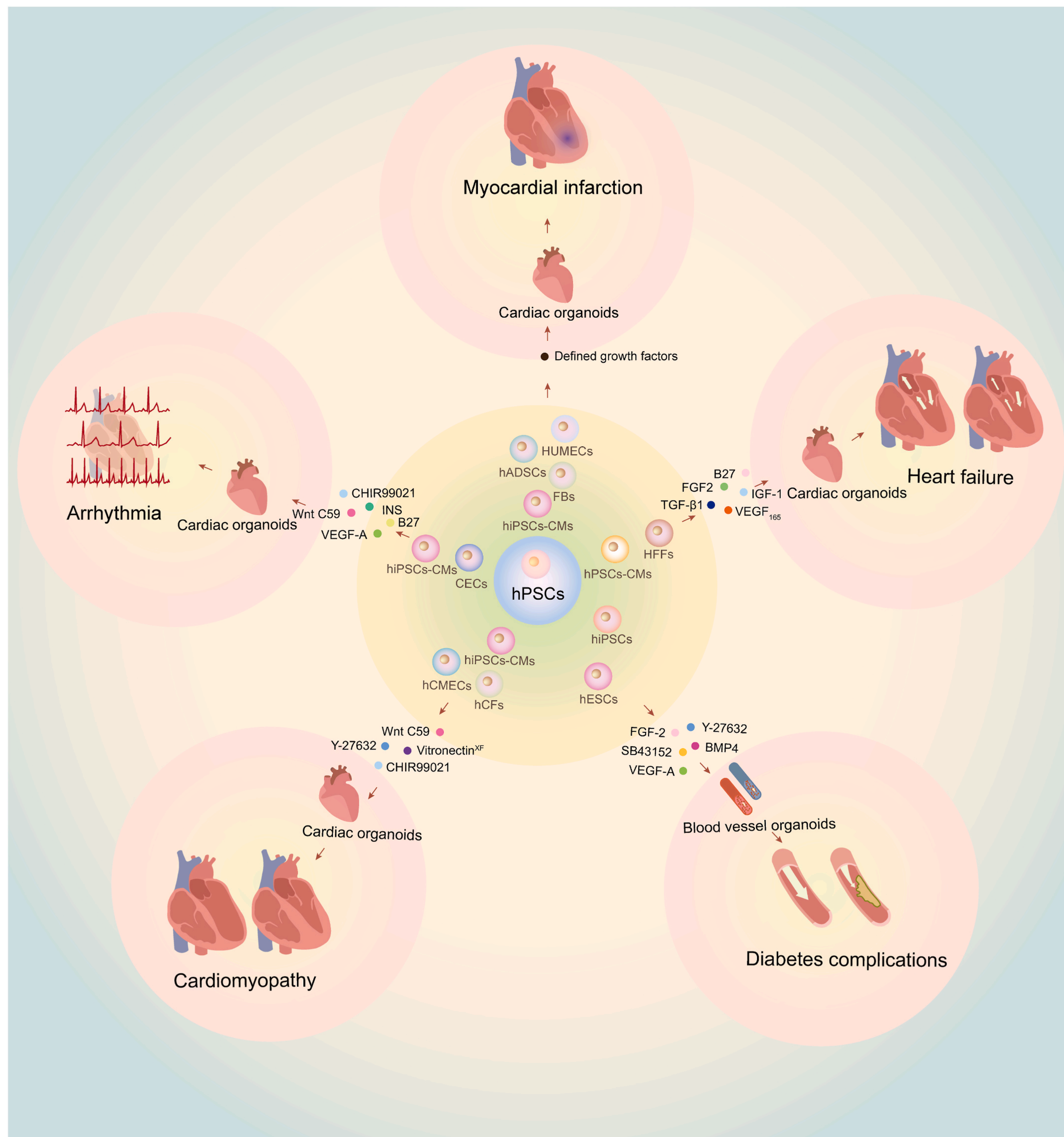

Fig. 3. Human cardiovascular organoids in CVDs modeling. Human cardiovascular organoids have played a significant role in modeling CVDs. Co-culturing hPSCs with other human-derived cells, followed by the application of specific growth factors, generates corresponding human organoids for CVDs. Human cardiac organoids can be subjected to various treatments to mimic myocardial infarction, heart failure, cardiomyopathy, and arrhythmia. Human blood vessel organoids can be utilized to model diabetes-related vascular complications.

progression and drug responses in cardiac organoids. These models help in understanding complex biological processes and in identifying potential therapeutic targets [83]. AI-driven analysis helps in identifying disease mechanisms and potential interventions [83]. The combination of automation and AI is significantly enhancing the precision, efficiency, and scalability of organoid-based cardiovascular research, which is essential for cardiovascular disease modeling, drug development, and personalized medicine.

3. Cardiovascular organoids in CVDs modeling

Cardiovascular organoids offer several advantages over traditional 2D cell cultures and animal models, including enhanced physiological relevance, organ-specific functionality, and the ability to incorporate patient-specific characteristics [84], which allows for the identification of intricate communication interactions among ECs, smooth muscle cells (SMCs), pericytes, FBs and myocardial cells, providing a more insightful understanding of their cooperative roles within the cardiovascular system [85]. Over the past few years, researchers began to utilize human CVOs derived from iPSCs or other sources to reproduce specific aspects of in vivo heart and blood vessels to model various CVDs, including myocardial infarction, heart failure, diabetes-related vascular complications, cardiomyopathy, and arrhythmia.

3.1. Myocardial infarction

Myocardial infarction (MI) occurs when cholesterol and other substances (plaque) accumulate on the arterial walls, narrowing or blocking the coronary arteries that supply oxygen and nutrients to the heart, resulting in myocardial damage or the death of heart tissue [86]. The fabrication of human COs has significantly advanced our understanding of MI-induced injury. The earliest documented CVD model using hCOs focused on replicating MI. In this model, hiPSC-CMs, human cardiac ventricular fibroblasts, HUVECs, and human adipose-derived stem cells were combined with specific growth factors to generate hCOs (Table 1) [66]. These organoids were subsequently subjected to a hypoxia chamber within an incubator, maintaining a gas concentration of 10 % O_2 with 1 μM of norepinephrine for 10 days to establish an MI model (Fig. 3) [66]. To create an in vitro 3D model replicating post-MI myocardial tissue, Richards et al. developed a fusion of hCOs with non-inherited pathogenic MI factors, which involved leveraging the distinct zones within MI, namely the infarct-border-remote zones, to establish a gradient delineating an apoptotic center, dysfunctional interior, and functional edge within the cardiac organoids [66]. This dynamic gradient was achieved by capitalizing on nutrient transfer, particularly oxygen diffusion, within the intricate 3D microstructure of the organoids, further sustained by continuous stimulation (Fig. 3) [66]. The generation of hCOs involved a comprehensive strategy, incorporating hiPSC-CMs, human ventricular cardiac FBs, and HUVECs to faithfully represent major cardiac cell types (Table 1) [87]. Subsequent spheroid treatment with a 6-h nutrient and oxygen reduction protocol effectively simulated ischemia, devoid of reperfusion. Under these ischemic conditions, a notable regulation of interleukin 6 (IL6) and vascular endothelial growth factor A (VEGF-A) expressions ensued, both distinctive markers of ischemic heart events [87]. The elevated IL6 secretion was attributed to the presence of human cardiac fibroblasts (HCFs), evidenced by the highest levels of IL6 gene expression observed in HCFs spheroids [87]. This finding highlights the importance of co-culture in attaining accurate modeling. In adult hearts, regenerative capacity following ischemia is limited, characterized by poor revascularization and lack of myogenesis [87]. While cardiac regeneration has been observed in zebrafish and neonatal mice, the full regenerative potential of MI-induced heart injury remains uncertain. Advances in pluripotent stem cell differentiation and tissue engineering have facilitated the creation of hCOs that resemble fetal heart tissue, providing an essential model for repairing MI-induced heart injury. However, whether this regenerative potential extends to the MI model remains to be investigated in hCOs following exposure to MI-induced injury.

3.2. Heart failure

Heart failure is characterized as a syndrome wherein a multitude of factors contribute to compromised cardiac pumping function, ultimately leading to insufficient cardiac output to meet the fundamental metabolic needs of the entire body [88]. A research team has successfully established an innovative method for engineering human myocardium (EHM), utilizing differentiated myocardial cells and FBs derived from hPSCs under serum-free conditions (Fig. 3) (Table 1) [69]. This approach has demonstrated the generation of hCOs with structural and functional properties closely resembling those of postnatal myocardium. In this study, human foreskin fibroblasts and hPSC-CMs were employed to generate hCOs with growth factors such as fibroblast growth factor 2 (FGF2), transforming growth factor beta-1 (TGF-β1), $VEGF_{165}$, insulin-like growth factor-1 (IGF-1), and B27. L-Norepinephrine hydrochloride and endothelin-1 were prepared in distilled water containing 200 μmol/L ascorbic acid to treat hCOs with the indicated concentrations for 7 days to establish a heart failure model [69]. The engineered EHM-hCOs exhibit fundamental structural and functional characteristics of the myocardium, including rod-shaped CMs, systolic twitching forces akin to those observed in actual postpartum myocardium, a positive force-frequency-response, and advanced molecular maturation, as confirmed by transcriptomic analysis [69]. Moreover, EHM-hCOs demonstrated susceptibility to chronic catecholamine toxicity, which led to contractile dysfunction, cardiomyocyte hypertrophy, cell death, and the release of N-terminal fragments of proBNP, providing valuable insights into heart failure, particularly the effects of chronic catecholamine exposure on cardiac function and cellular dynamics [9,69].

3.3. Diabetes-related vascular and cardiac complications

Diabetes significantly increases the risk of cardiovascular complications, including both macrovascular and microvascular issues such as diabetic kidney disease, diabetic retinopathy, neuropathy, and congenital heart defects [89]. Whereas, the inherent differences between conventional animal models and humans hinder the therapeutical translation for the treatment of diabetes-related vascular complications. Wimmer et al. achieved a significant breakthrough by utilizing human iPSCs and relevant growth factors such as BMP4, VEGF-A, and FGF-2 to develop self-organizing 3D-BVOs that faithfully mimic diabetic vasculopathy (Fig. 3) [27]. These hBVOs comprised ECs and pericytes, autonomously forming capillary networks surrounded by a basement membrane. Upon transplantation into mice, they were able to form stable vascular trees including arteries, arterioles, and venules. Culturing hBVOs in vitro under conditions of hyperglycemia resulted in the thickening of the vascular basement membrane. In vivo, when subjected to a diabetic environment in mice, hBVOs replicated the microvascular alterations observed in individuals with diabetes (**Table 1**) [27]. Furthermore, key contributors to diabetic vasculopathy in human blood vessels, such as DLL4 and NOTCH3, were identified. Thus, hBVOs derived from human stem cells can effectively reproduce the structure and function of human blood vessels, providing flexible systems for modeling and identifying factors that influence hyperglycemia-related vascular diseases [27,90]. Nevertheless, the further application of hBVOs for diabetes-related vascular complications still warrants extensive investigation. The incidence of pregestational diabetes (PGD)-induced congenital heart defects (CHD) is rising alongside the global diabetes epidemic, yet the underlying mechanism still remains unclear. In 2021, Lewis-Israeli et al. reported that self-organizing human COs with well-organized multi-lineage cardiac cell types, atrioventricular specification, and robust functional activity. These hCOs, based on hPSCs and developed using a three-step Wnt

signaling modulation strategy with chemical inhibitors and growth factors, circumvent many technical and ethical challenges, providing a valuable system for modeling PGD-induced CHD (Table 1) [8,67]. Furthermore, by employing this sophisticated hCO system to model embryonic heart development under conditions mimicking pregestational diabetes, Kostina et al. elucidated that endoplasmic reticulum stress and dyslipidemia contribute to diabetic embryonic cardiomyopathy (Table 1) [71].

3.4. Cardiomyopathy

Cardiomyopathy is a myocardial disease characterized by abnormalities in cardiac mechanical and electrical activity, leading to inappropriate hypertrophy or dilation of the ventricles [91]. Human cardiovascular organoids have emerged as valuable tools for studying cardiomyopathy modeling, and offer a more physiologically relevant model for investigating the structural and functional changes that occur in the heart during cardiomyopathy.

Hypertrophic cardiomyopathy (HCM) is a genetic heart disease characterized by structural abnormalities and impaired contractile function. In 2019, Prondzynskicreated et al. induced patient-derived hiPSC-CMs to form COs that faithfully replicated various characteristics of HCM, including hypertrophy, disorganized myofibrils, heightened contractility, compromised relaxation, increased sensitivity to myofilament Ca^{2+}, prolonged action potential duration, and augmented L-type Ca^{2+} current [92]. Buono et al. further demonstrated the generation of COs using a triculture method involving hiPSC-CMs derived from both healthy individuals and cardiomyopathy patients, human cardiac microvascular endothelial cells (HCMECs), and HCFs. During the organoid culture process, growth factors such as VitronectinXF, Y-27632, Wnt-C59, and CHIR99021 were added to promote the formation of hCOs (Fig. 3) [93]. This approach aimed to evaluate the potential of organoids as 3D models for accurately representing the phenotypic characteristics of both healthy and cardiomyopathic hearts. They observed distinct differences in the structural and beating behavior of the hCOs depending on whether the hiPSC-CMs were derived from healthy individuals or cardiomyopathy patients (Table 1) [93].

Duchenne muscular dystrophy (DMD) cardiomyopathy, a genetic disorder, refers to the dysfunction of the heart muscle in individuals with DMD. However, the underlying mechanisms of its development remain poorly understood, as current cellular and animal models fail to fully recapitulate the phenotypic features of human DMD cardiomyopathy. Interestingly, Marini et al. successfully developed hCOs using iPSCs derived from patients and isogenic-corrected controls. These DMD-COs, which display DMD-related cardiomyopathy features, allow researchers to explore the development and progression of DMD-related cardiomyopathy over extended culture periods (Table 1) [94]. Myocarditis is a frequent contributor to cardiomyopathy, particularly in individuals diagnosed with COVID-19 [95]. Recently, Wang et al. established human iPSCs-derived 3D hCOs with direct infection of SARS-CoV-2 and Omicron BA.5 strains to recapitulate COVID-19 myocarditis, which provided an alternative approach for investigating the pathophysiological processes of SARS-CoV-2-induced myocarditis (Table 1) [70]. These findings highlight the potential of organoids as valuable tools for modeling various types of cardiomyopathies and for designing patient-specific treatments.

3.5. Arrhythmia

Arrhythmia, characterized by pathological irregularities in heart rate or rhythm, can significantly impair heart function and elevate the risk of clotting, heart failure, and even sudden cardiac death [96]. The use of hCOs has extended to modeling structural arrhythmic diseases, where these organoids produce both spontaneous and induced action potentials, demonstrating elevated conduction velocities compared to 2D models [97]. Shinnawi et al. pioneered a study incorporating patient-specific hiPSC-CMs, CRISPR/Cas9 genome editing, and organoids technology to investigate short QT syndrome (SQTS), an inherited arrhythmogenic disorder (Table 1) [68]. A patient-specific iPSC line was derived from an individual with a 270 ms QT interval on the electrocardiogram, and CRISPR/Cas9 was employed to correct a heterozygous missense mutation (N588K) in the S5-P loop region of the KCNH2-encoded hERG channel [68]. These findings demonstrated the potential of human iPSCs to model SQTS in vitro, providing novel mechanistic insights into pathological QT shortening. However, modeling complex electrophysiological phenomena such as re-entrant arrhythmias remained challenging at the cellular level. To overcome this, hiPSC-CMs were seeded into 3D macrostructures, creating large-scale (5-mm) cardiac cell sheets that formed a functional syncytium with consistent action potential propagation [68]. Notably, these cell sheets (hCOs), generated from SQTS hiPSC-CMs, exhibited shorter tissue wavelengths under electrical stimulation and sustained re-entrant arrhythmias in the form of spiral waves.

Additionally, another study proposed an organoid structure to reproduce arrhythmic events as seen in arrhythmia patients due to the ability of iPSC to model SQTS at the cellular level [97,98]. Recently, Ghosheh et al. reported the generation of multi-chambered self-paced vascularized hCOs formed under anisotropic stress, demonstrating their applicability for inducing cardiac arrhythmia. In this approach, CMs are differentiated from hiPSC with Wnt-59C, CHIR99021, INS, B27, VEGF-A for 10 days, dissociated and mixed with rat primary HCMECs. The cell suspension was seeded into a microwell for geometric confinement, and COs were formed within 4 days, developing multiple chambers under anisotropic stress following 25 days in culture (Fig. 3) (Table 1) [99]. A microphysiological system, incorporating sensors into the hCOs, enabled the concurrent monitoring of oxygen uptake, extracellular field potentials, and cardiac contractions at a resolution greater than 10 Hz, revealing 1 Hz cardiac respiratory cycles that are coupled to the electrical activity driven by mitochondrial calcium oscillations [99]. This study demonstrated that pharmaceutical agents (e.g., mitoxantrone) or genetic disruption of this coupling can effectively model arrhythmia by utilizing hCOs.

4. The prospects of cardiovascular organoids for CVDs therapy

In recent years, the development of cardiovascular organoids has greatly advanced our understanding of CVD pathogenesis by replicating key features of the human cardiovascular system. Additionally, cardiovascular organoids provide a valuable platform for studying CVD therapies, including drug development, toxicity testing, personalized medicine, regenerative medicine, and organ transplantation.

4.1. Cardiovascular drugs development and toxicity testing

Approximately 90 % of newly developed pharmaceuticals encounter regulatory approval challenges during phase II and III clinical trials. This failure is primarily due to a lack of therapeutic efficacy rather than safety concerns, often stemming from inadequate validation of drug targets. Current target validation approaches rely heavily on animal models and in vitro assays, which frequently fail to accurately replicate human disease conditions due to significant species differences in functional and biological features [100]. Unlike conventional heart models, human cardiovascular organoids present a promising solution to this translational barrier by providing an unlimited source of hCVOs for drug development and toxicity testing.

Mills. et al. developed a high-throughput bioengineered human pluripotent stem cell-derived hCOs system that exhibited functional contractile tissue with biological characteristics closely resembling native cardiac tissue, including mature and cell-cycle-arrested CMs (Fig. 4) (Table 1) [101]. Using these hCOs, there were two small molecules identified to be able to induce cardiomyocyte proliferation without detrimental impacts. In addition, high-throughput proteomics

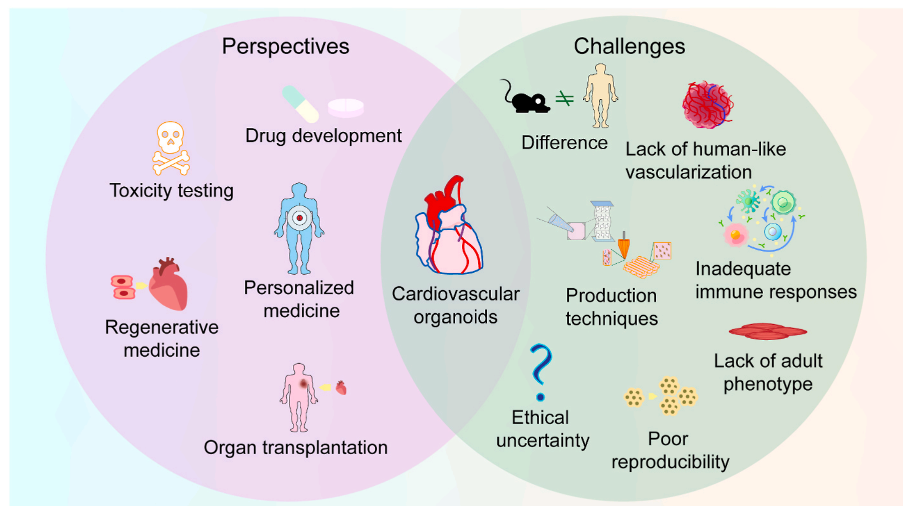

Fig. 4. The prospects of human cardiovascular organoids in CVDs therapy and their developmental challenges. Human organoid technology offers promising applications in cardiovascular research, including drug screening, toxicity testing, personalized medicine, regenerative medicine, and transplantation therapy. However, it also faces several challenges, such as the gap between animal and human models, lack of human-like vascularization, inadequate immune responses, production technique challenges, lack of adult phenotype, poor reproducibility, and ethical uncertainties.

in hCOs uncovered the cooperative activation of both the mevalonate pathway and a cell-cycle network by pro-proliferative compounds [101]. Cell-cycle reentry in hCOs was contingent on the mevalonate pathway, as demonstrated by the attenuation of pro-proliferative effects with statin-mediated inhibition of this pathway [101]. This study highlights the effectiveness of hCOs in assisting in pro-regenerative drug development for cardiac diseases, providing insights into underlying biological mechanisms while mitigating potential adverse side effects. In another study using hiPSC-derived hCOs composed of FBs, hiPSC-CMs, and hiPSC-derived ECs, 2-Cl-C.OXT-A (COA-Cl), a novel synthetic adenosine analog, was found to increase the beating rate and fractional area change by inhibiting phosphodiesterase activity, independent of sphingosine-1-phosphate receptor 1 and adenosine A1 receptor activity (**Table 1**) [102], suggesting that COA-Cl could potentially serve as a future inotropic agent to enhance cardiac contraction. Patients infected with COVID-19 frequently exhibit cardiac dysfunction and damage, increasing their risk of mortality. In the hCOs infected with SARS-CoV-2, bromodomain-containing protein 4 was demonstrated to be activated and involved in viral response, and inhibitors targeting the bromodomain and extraterminal family effectively suppressed the gene expression of viral response genes, angiotensin-converting enzyme 2 (ACE2), and SARS-CoV-2 infection in CMs, offering potential therapeutic candidates to mitigate COVID-19-mediated cardiac damage [44]. In a groundbreaking study utilizing heart organoids generated through a self-organization-driven developmental induction strategy, Volmert et al. demonstrated that ondansetron, a 5-HT3 receptor antagonist and antiemetic commonly used for treating nausea and vomiting, significantly disrupted critical stages of ventricular heart development, which were reminiscent of clinical phenotypes epidemiologically associated with ondansetron use, specifically ventricular septal defects [41]. A type of hCOs incorporating an oxygen-diffusion gradient and stimulated with the neurotransmitter noradrenaline can recapitulate the structural features of the human heart post-MI, which can be used to model hypoxia-enhanced doxorubicin cardiotoxicity, thereby making them valuable tools for drug screening and development [66]. By micropatterning and differentiating hiPSCs, Hoang et al. have engineered spatially organized hCOs, featuring contracting cardiomyocytes centrally surrounded by stromal cells arranged along the pattern perimeter (**Table 1**) [103]. These organoids have been successfully used to quantify the embryotoxic potential of nine pharmaceutical compounds, demonstrating significant potential as an in vitro platform for investigating drug-induced cardiac developmental toxicity (Fig. 4) [103]. The synergistic interplay of biochemical and biophysical cues promotes self-organized lineage specification, in turn leading to the formation of a human cardiac microchamber confined by the pattern geometry (**Table 1**) [32]. These precisely defined human cardiac microchambers could serve as a valuable platform for investigating cardiac drug-induced developmental toxicity [32]. Additionally, studies using diabetic BVOs revealed that only the γ-secretase inhibitor DAPT effectively reduced collagen type IV expansion, and administering DAPT to diabetic BVOs restored endothelial cell proliferation [27]. Moreover, the hBVOs from iPSCs, comprising pericytes and ECs, offer a versatile platform for conducting pharmacological screening and evaluating the vascular toxicity of small-molecule drugs [28].

4.2. Cardiovascular personalized medicine

Personalized medicine, with a focus on treating illnesses based on individual patient profiles, offers the potential to significantly improve patient outcomes and reduce healthcare costs [104]. Recently, researchers integrated the technology of PSCs derived from patients with CVDs and organoid modeling to exemplify the forefront of personalized medicine, offering more effective and targeted treatments to improve patient outcomes (Fig. 4) [105]. To accurately replicate patient-specific disease conditions, an innovative technique involved the integration of HCMECs, HCFs, and CMs derived from the hiPSCs of individuals with cardiomyopathy were adopted to generate hCOs through a triculture approach [93]. These patient-derived hCOs facilitate personalized medical interventions by assessing the impact of medications on the human cardiovascular system and gene expression patterns using patient-specific iPSCs [17], which play a crucial role in advancing cardiovascular precision medicine by enabling the unlimited generation of patient-specific somatic cells. The utilization of patient-specific iPSC--derived cardiovascular cells presents an unprecedented opportunity to identify novel therapeutic targets and screen potential treatments for CVDs [106]. In addition, the creation of hBVOs from patient-derived iPSCs with hereditary vascular disorders offers promising models for studying rare genetic vascular diseases [28].

4.3. Cardiovascular regenerative medicine

The regenerative capacity of the adult human heart is notably limited, with a cardiomyocyte renewal rate of approximately 1 % per year [107]. As such, restoring damaged myocardium by promoting the

proliferation of resident CMs in vivo or by directly introducing new CMs to replace necrotic tissue could be a potential strategy for cardiac regeneration (Fig. 4) [108–110]. The development of cardiovascular organoid culture technologies is leading to substantial progress in facilitating tissue regeneration. In a groundbreaking study, researchers employed a combination of human embryonic stem cell-derived cardiac progenitor cells, mesenchymal stem cells, and HUVECs on hydrogels to create 3D hCOs encapsulated in collagen I (**Table 1**) [111]. These hCOs were then transferred to a 3D-printed polylactic acid basket and directly sutured into the peritoneal cavity of a nude mouse. Remarkably, one-month post-transplantation, the CMs within the hCOs demonstrated increased maturity at both transcriptional and ultrastructural levels [111]. Additionally, the hCOs featured a primitive vascular system supporting energy, oxygen exchange, and waste material removal. The prevascularized hCOs also created a microenvironment rich in angiogenic growth factors, facilitating the migration of host ECs into the organoids [111]. hBVOs, composed of ECs and pericytes, were derived from iPSCs. Following mesoderm induction, endothelial networks formed through vessel sprouting, maturing into stable blood vessels supported by pericytes and surrounded by a basement membrane, representing a significant breakthrough for the regeneration of human organs [28].

4.4. Cardiovascular organs transplantation

Organ transplantation has long been a cornerstone of medical treatment, but the ongoing shortage of available organs, coupled with the growing number of patients in need, highlights the urgent need for alternative solutions. The emergence of hPSCs has significantly advanced the development of human organoids, offering a promising avenue for transplantation in target organs [112–114]. Notably, organoids generated from patients and donors seldom encounter graft rejection, representing a transformative application in addressing the organ shortage crisis (Fig. 4) [43]. The successful transplantation of heart and vascular organoids into live animals indicates the potential for future clinical applications, including transplantation of these organoids into humans (**Table 1**) [27,114]. In a clinical study, allogenic hiPSC-CMs patches without any genetic mutations in cancer-related genes were implanted into the left ventricle epicardium of a patient with ischemic cardiomyopathy via thoracotomy [115]. The clinical symptoms improved 6 months after surgery, without any major adverse events, suggesting that the patches were well-tolerated. Furthermore, changes in the wall motion in the transplanted site were recovered without any major adverse events [116]. Recently, Tan et al. demonstrated that hCOs modified with electrically conductive silicon nanowires substantially increased the therapeutic effectiveness of hPSC-CMs in treating hearts with infarction (**Table 1**) [114]. These nanowire-incorporated hCOs were incorporated with hPSC-CMs, non-myocyte supporting cells, and electrically conductive silicon nanowires. Following transplantation into rat hearts with ischemia/reperfusion-induced injury, the nanowired COs significantly enhanced the contractile maturation of engrafted hPSC-CMs, facilitated robust recovery in cardiac function, and mitigated maladaptive left ventricular remodeling [114]. These innovative approaches underscore the promising potential of stem cell-derived organoids in addressing transplantation challenges and advancing therapeutic strategies for CVDs.

5. Challenges in the development and application of human cardiovascular organoids

Human cardiovascular organoids have emerged as a promising model system that can complement conventional animal models, providing valuable tools for elucidating CVDs pathogenesis and drug development. However, numerous limitations and unanswered questions remain to be addressed.

5.1. The gap between single tissue environment and the entire human body environment

The progression of organoid technology is intricately intertwined with the concurrent utilization of conventional animal models and cell culture techniques. In recent years, cardiovascular organoid technology has been extensively employed to investigate human CVDs, significantly advancing our comprehension in cardiovascular pathology. In particular, COs have proven invaluable in modeling hereditary heart abnormalities and disorders, which involve the incorporation of hPSCs into Matrigel, inducing the formation of intricate hCOs through the Wnt pathway [36]. However, CVOs are generated in ex vivo and cannot completely and accurately replicate the entire human body environment and real pathophysiological conditions. Despite the inherent limitations of animal models stemming from interspecific variations in drug sensitivity and cell structure, CVOs cannot entirely replace their role in basic and clinical research of CVDs (Fig. 4). hBVOs, for instance, have demonstrated the ability to penetrate human cerebral organoids, forming a vessel-like system [117]. Nevertheless, for comprehensive drug evaluation, the entire biological environment must be considered—something that organoids alone cannot fully achieve [118].

5.2. The vascularization is non-human without adequate immune responses

Vascularization during the generation of CVOs is a key technique for replicating the human cardiovascular system and supporting the long-term culture of organoids, which is also vital for both fundamental research and clinical applications (Fig. 4) [16]. Whereas, achieving human-like vascularization remains a significant challenge. To ensure human-like vascularization, ECs derived from human iPSCs or human embryonic stem cells (hESCs) should be employed, as these cells form the lining of blood vessels. Additionally, human pericytes and smooth muscle cells can help support vascular stability and mimic human vasculature more closely. Furthermore, current COs primarily consist of CMs, ECs, and FBs, but the absence of immune cells compromises their ability to reproduce the immune environment of the cardiovascular system [28]. It is widely recognized that dysregulation of immunity and immune responses is strongly correlated with the pathogenesis of CVDs [119]. To overcome adequate immune responses in CVOs, one approach involves incorporating human immune cells, such as macrophages, dendritic cells, or T-cells, into CVOs, which helps to create a more physiologically relevant immune environment that closely replicates human inflammatory responses.

5.3. Lack of adult phenotype

Tissue maturity is critical for understanding organ development and replicating physiological conditions in CVDs research. Unfortunately, only a limited number of stem cell-derived hCOs and hBVOs are capable of developing in mature and complete organs with adult phenotype (Fig. 4). The majority of organoids only replicate the early stages of organogenesis. For instance, researchers have successfully generated brain organoids by seeding embryoid bodies from pluripotent stem cells, which persisted for over ninety days but only closely mimicked fetal brain development [120]. Similar limitations are also observed in CVOs, which only can capture the early stages of organogenesis. To get rid of these constraints, ongoing efforts are focused on refining differentiation protocols for various cell types to promote the fabrication of mature CVOs.

5.4. Challenges of production techniques

Presently, the production of well-organized CVOs faces several challenges, including heterogeneity, lack of functional and structural fidelity, immuration, and scalability. Encouragingly, as the emergences

and advances of 3D printing, microfluidics chip, organoids-on-chip, and 4D printing, these challenges are doomed to be tackled. 3D printing allows for the precise placement of different cell types within the CVOs, facilitating the creation of customizable and well-organized cardiac and vascular structures and mimicking the spatial organization of the cardiovascular system (Fig. 4). Human chambered organoids, replicating the intricate geometric structure of the heart with chambers, electromechanical function, and large vessels, have been successfully produced using 3D bioprinting technology [121]. Microfluidic chip, is a tiny and integrated system that manipulates fluids on the scale of microliters or nanoliters, allowing for the creation of precise environments for cellular interactions and facilitating controlled experiments in tissue engineering and organoid development [122]. A 3D printing-based platform combined with a microfluidic chip has been developed through co-development efforts, facilitating a sequential and developmentally matched co-culture system. This system demonstrates that hPSC-derived pericytes and ECs sprout and self-assemble into organized vascular networks, leading to the formation of integrated neurovascular organoids [31]. Organoids-on-chip, combines the advantages of organoid cultures with microfluidic systems to create dynamic, 3D models of human organs, which allows researchers to study organ development, function, and disease in a more physiologically relevant environment [123]. Recently, a novel microfluidic platform integrating functionalized vascularized organoids-on-chip has been successfully established to monitor the formation of endothelial networks surrounding mesenchymal and pancreatic islet spheroids, as well as blood vessel organoids derived from PSCs, which represents a promising organ-on-chip model for vascularizing diverse biological 3D tissues [60]. 4D bioprinting, a next-generation bioprinting technology, extends traditional 3D bioprinting by incorporating the "time" dimension, which enables the creation of objects that can dynamically alter their shape in response to external stimuli [124]. With the goal of developing in vitro models that closely resemble native tissue structures, 4D bioprinting facilitates dynamic, structural, and cellular changes within a tissue over time, addressing the static limitations of 3D bioprinting [124]. Moreover, rapid advancements in 3D and 4D printing technology facilitate the design, fabrication, and translation of results from 3D cell culture models, which can also serve as innovative tools to promote the development and propagation of organoids-on-chip systems. Hence, the future development and effective production of CVOs that closely resemble in vivo human organs will require the synergistic integration of multiple techniques.

5.5. Poor reproducibility

Reproducibility is also a crucial factor in their ongoing investigation and application of CVOs for modeling CVDs and drug testing (Fig. 4). The repeatability of organoids refers to the ability of PSCs to consistently generate organoids with identical cell composition, structure, and function under specified conditions [125]. Several key elements influence the reproducibility of data obtained from CVOs, including their architecture, cellular composition, and scalability of production. Currently, the reproducibility of CVOs produced for CVDs therapy is incompetent, necessitating further research and optimization to establish standardized protocols to improve reproducibility [126]. Factors such as assay type, complexity, and the source of initial cells—whether primary stem cells derived from fetal or adult tissues, or well-characterized cell lines—influence to the repeatability of CVOs [127]. Achieving consistent size, shape, architecture, cellular composition, and function is essential to ensure the reliability of organoid-based research and therapeutic applications for CVDs research [128]. To improve the reproducibility of CVOs, the group led by Aguirre and Zhou has successfully developed high-content platforms and optical coherence tomography (OCT) systems for comprehensive organoid evaluation [41,73,129]. OCT is a rapid, non-destructive, and label-free imaging technique that utilizes naturally backscattered light to generate high-contrast images at the interfaces of different materials [130]. A customized spectral-domain optical coherence tomography (SD-OCT) system can be configured to monitor the developmental dynamics of chamber structures and beating patterns in hHOs through integrated OCT and calcium imaging [73,129]. A dual-modality imaging system combining the OCT and fluorescence microscopy has been developed, which allowed us to monitor in vitro beating of hHOs [129]. Also, the OCT microscopy system can be employed to provide detailed characterization of chamber structures in live organoids over time and to measure the growth and monitor the dynamics of chamber development during developmental induction conditions for high-content screening applications [41]. Furthermore, as the emergence of 3D bioprinting-generated organoids utilizing a low-cost and customizable 3D bioprinting system [121,131,132], 3D bioprinting system can contribute to enhancing the production and reproducibility of cardiovascular organoid production by providing consistent structural design, precise cell placement, scalability, automation, and a customizable microenvironment.

5.6. Ethical uncertainty

In organoid research, informed consent is one of the most frequently debated ethical issues, playing a critical role in the creation and use of organoids [133]. A number of researchers have voiced concerns about the use of organoids for basic and clinical studies of human diseases, including CVDs [134]. Bredenoord et al. have emphasized several ethical challenges, including the evaluation of donor agreements, long-term use, preservation, and the responsibility to communicate clinically significant findings to patients. Additionally, the use of organoids for personalized drug testing, especially when bridging the gap between preclinical and clinical development and incorporating gene editing, may introduce additional ethical uncertainties (Fig. 4) [135]. To address these ethical uncertainties, researchers should ensure robust consent processes, transparency in the use and storage of biological materials, and international collaboration to standardize ethical guidelines. Concerning the informed consent, researchers should design clear and comprehensive consent forms that outline the purpose, procedures, potential risks, and uses of CVO research, and how participant data and samples will be managed. This should also include information on any potential commercial applications or partnerships. For long-term studies, it is advisable to obtain re-consent or provide updates to participants, particularly if the research purposes, procedures, or potential risks change. Moreover, consent processes should respect the cultural and personal beliefs of participants, ensuring that the consent is informed, voluntary, and documented according to ethical standards. To maintain transparency regarding the use and storage of biological materials, researchers should inform participants about how their biological materials are stored, accessed, and utilized over time. Providing access to a database where participants can track the usage of their samples may enhance transparency. Rigorous data privacy measures should be implemented to protect participant information, including anonymization or pseudonymization of samples and data. Participants should also be informed about any collaborations or data-sharing agreements with third-party researchers, institutions, or companies, especially for global research initiatives. With respect to international collaboration, establishing unified protocols with international research bodies for consent, data sharing, and storage practices that comply with various jurisdictions is essential for ensuring ethical consistency. Additionally, researchers should form partnerships with ethics committees and regulatory bodies worldwide to periodically review and update ethical guidelines. By implementing these strategies, researchers can navigate the ethical complexities associated with CVOs research more effectively, strengthening trust and compliance within the scientific community and among participants.

6. Concluding remarks

Human cardiovascular organoids enable the in vitro generation of multicellular tissues that closely replicate the architecture and functionality of human cardiovascular organs, offering significant advantages for preclinical research and transplantation studies related to cardiovascular diseases (CVDs). In recent years, CVOs have been extensively studied to deepen our understanding of CVD pathogenesis and to facilitate the development and testing of CVD drugs. These organoids provide a more accurate prediction of drug effects in the human body, thereby reducing risks and costs associated with drug development. Despite the development and clinical application of CVOs currently facing several challenges, CVOs are doomed to be novel and promising tools for cardiovascular research and clinical translation. Continued optimization and validation are essential for unlocking their full potential.

CRediT authorship contribution statement

Jinli Li: Writing – review & editing, Writing – original draft, Data curation. **Yang Li:** Writing – review & editing. **Guangtao Song:** Supervision, Formal analysis. **Haiying Wang:** Supervision, Investigation. **Qing Zhang:** Investigation. **Min Wang:** Investigation. **Muxue Zhao:** Investigation. **Bei Wang:** Investigation. **HuiGuo Zhu:** Investigation. **Liu Ranzhi:** Investigation. **Qiang Wang:** Supervision, Project administration, Funding acquisition. **Yuyan Xiong:** Writing – review & editing, Supervision, Project administration, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mtbio.2024.101396.

Data availability

No data was used for the research described in the article.

References

[1] O. Gaidai, Y. Cao, S. Loginov, Global cardiovascular diseases death rate prediction, Curr. Probl. Cardiol. 48 (5) (2023), https://doi.org/10.1016/j.cpcardiol.2023.101622.

[2] G. Rossi, A. Manfrin, M.P. Lutolf, Progress and potential in organoid research, Nat. Rev. Genet. 19 (11) (2018) 671–687, https://doi.org/10.1038/s41576-018-0051-9.

[3] J. Kim, B.-K. Koo, J.A. Knoblich, Human organoids: model systems for human biology and medicine, Nat. Rev. Mol. Cell Biol. 21 (10) (2020) 571–584, https://doi.org/10.1038/s41580-020-0259-3.

[4] C. Zaragoza, C. Gomez-Guerrero, J.L. Martin-Ventura, L. Blanco-Colio, B. Lavin, B. Mallavia, C. Tarin, S. Mas, A. Ortiz, J. Egido, Animal models of cardiovascular diseases, J. Biomed. Biotechnol. 2011 (2011) 1–13, https://doi.org/10.1155/2011/497841.

[5] C. St Hilaire, Cardiovascular organoids/3D models review series: an introduction, Circ. Res. 132 (4) (2023) 481–482, https://doi.org/10.1161/circresaha.123.322561.

[6] A. Beauchamp, Y. Yee, B.C. Darwin, A. Raznahan, R.B. Mars, J.P. Lerch, Whole-brain comparison of rodent and human brains using spatial transcriptomics, Elife 11 (2022), https://doi.org/10.7554/eLife.79418.

[7] A. Wessels, D. Sedmera, Developmental anatomy of the heart: a tale of mice and man, Physiol. Genomics 15 (3) (2003) 165–176, https://doi.org/10.1152/physiolgenomics.00033.2003.

[8] Y.R. Lewis-Israeli, A.H. Wasserman, M.A. Gabalski, B.D. Volmert, Y. Ming, K. A. Ball, W. Yang, J. Zou, G. Ni, N. Pajares, X. Chatzistavrou, W. Li, C. Zhou, A. Aguirre, Self-assembling human heart organoids for the modeling of cardiac development and congenital heart disease, Nat. Commun. 12 (1) (2021), https://doi.org/10.1038/s41467-021-25329-5.

[9] D. Zhao, W. Lei, S. Hu, Cardiac organoid - a promising perspective of preclinical model, Stem Cell Res. Ther. 12 (1) (2021), https://doi.org/10.1186/s13287-021-02340-7.

[10] C. Corro, L. Novellasdemunt, V.S.W. Li, A brief history of organoids, Am. J. Physiol. Cell. Physiol 319 (1) (2020) C151–C165, https://doi.org/10.1152/ajpcell.00120.2020.

[11] H. Clevers, Modeling development and disease with organoids, Cell 165 (7) (2016) 1586–1597, https://doi.org/10.1016/j.cell.2016.05.082.

[12] M.B. Rookmaaker, F. Schutgens, M.C. Verhaar, H. Clevers, Development and application of human adult stem or progenitor cell organoids, Nat. Rev. Nephrol. 11 (9) (2015) 546–554, https://doi.org/10.1038/nrneph.2015.118.

[13] G. Schwank, B.-K. Koo, V. Sasselli, J.F. Dekkers, I. Heo, T. Demircan, N. Sasaki, S. Boymans, E. Cuppen, C.K. van der Ent, E.E.S. Nieuwenhuis, J.M. Beekman, H. Clevers, Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients, Cell Stem Cell 13 (6) (2013) 653–658, https://doi.org/10.1016/j.stem.2013.11.002.

[14] J. Drost, R.H. van Jaarsveld, B. Ponsioen, C. Zimberlin, R. van Boxtel, A. Buijs, N. Sachs, R.M. Overmeer, G.J. Offerhaus, H. Begthel, J. Korving, M. van de Wetering, G. Schwank, M. Logtenberg, E. Cuppen, H.J. Snippert, J.P. Medema, G. J.P.L. Kops, H. Clevers, Sequential cancer mutations in cultured human intestinal stem cells, Nature 521 (7550) (2015) 43–U329, https://doi.org/10.1038/nature14415.

[15] J. Drost, R. van Boxtel, F. Blokzijl, T. Mizutani, N. Sasaki, V. Sasselli, J. de Ligt, S. Behjati, J.E. Grolleman, T. van Wezel, S. Nik-Zainal, R.P. Kuiper, E. Cuppen, H. Clevers, Use of CRISPR-modified human stem cell organoids to study the origin of mutational signatures in cancer, Science 358 (6360) (2017) 234, https://doi.org/10.1126/science.aao3130.

[16] X.Y. Tang, S. Wu, D. Wang, C. Chu, Y. Hong, M. Tao, H. Hu, M. Xu, X. Guo, Y. Liu, Human organoids in basic research and clinical applications, Signal. Transduct. Target, Ther 7 (1) (2022) 168, https://doi.org/10.1038/s41392-022-01024-9.

[17] H. Kim, R.D. Kamm, G. Vunjak-Novakovic, J.C. Wu, Progress in multicellular human cardiac organoids for clinical applications, Cell. Stem, Cell 29 (4) (2022) 503–514, https://doi.org/10.1016/j.stem.2022.03.012.

[18] K. Salewskij, J.M. Penninger, Blood vessel organoids for development and disease, Circ. Res. 132 (4) (2023) 498–510, https://doi.org/10.1161/circresaha.122.321768.

[19] P. Horvath, N. Aulner, M. Bickle, A.M. Davies, E. Del Nery, D. Ebner, M. C. Montoya, P. Ostling, V. Pietiainen, L.S. Price, S.L. Shorte, G. Turcatti, C. von Schantz, N.O. Carragher, Screening out irrelevant cell-based models of disease, Nat. Rev. Drug Discov. 15 (11) (2016) 751–769, https://doi.org/10.1038/nrd.2016.175.

[20] F. Weinberger, I. Mannhardt, T. Eschenhagen, Engineering cardiac muscle tissue A maturating field of research, Circ. Res. 120 (9) (2017) 1487–1500, https://doi.org/10.1161/Circresaha.117.310738.

[21] G. Wang, M.L. McCain, L. Yang, A. He, F.S. Pasqualini, A. Agarwal, H. Yuan, D. Jiang, D. Zhang, L. Zangi, J. Geva, A.E. Roberts, Q. Ma, J. Ding, J. Chen, D.-Z. Wang, K. Li, J. Wang, R.J.A. Wanders, W. Kulik, F.M. Vaz, M.A. Laflamme, C. E. Murry, K.R. Chien, R.I. Kelley, G.M. Church, K.K. Parker, W.T. Pu, Modeling the mitochondrial cardiomyopathy of Barth syndrome with induced pluripotent stem cell and heart-on-chip technologies, Nat. Med. 20 (6) (2014) 616–623, https://doi.org/10.1038/nm.3545.

[22] J.T. Hinson, A. Chopra, N. Nafissi, W.J. Polacheck, C.C. Benson, S. Swist, J. Gorham, L. Yang, S. Schafer, C.C. Sheng, A. Haghighi, J. Homsy, N. Hubner, G. Church, S.A. Cook, W.A. Linke, C.S. Chen, J.G. Seidman, C.E. Seidman, Titin mutations in iPS cells define sarcomere insufficiency as a cause of dilated cardiomyopathy, Science 349 (6251) (2015) 982–986, https://doi.org/10.1126/science.aaa5458.

[23] M. Simian, M.J. Bissell, Organoids: a historical perspective of thinking in three dimensions, J. Cell Biol. 216 (1) (2017) 31–40, https://doi.org/10.1083/jcb.201610056.

[24] L. Zhu, K. Liu, Q. Feng, Y. Liao, Cardiac organoids: a 3D technology for modeling heart development and disease, Stem. Cell. Rev. Rep 18 (8) (2022) 2593–2605, https://doi.org/10.1007/s12015-022-10385-1.

[25] H. Naderi-Meshkin, V.A. Cornelius, M. Eleftheriadou, K.N. Potel, W.A. W. Setyaningsih, A. Margariti, Vascular organoids: unveiling advantages, applications, challenges, and disease modelling strategies, Stem Cell Res. Ther. 14 (1) (2023) 292, https://doi.org/10.1186/s13287-023-03521-2.

[26] S. Kusuma, Y.-I. Shen, D. Hanjaya-Putra, P. Mali, L. Cheng, S. Gerecht, Self-organized vascular networks from human pluripotent stem cells in a synthetic

matrix, Proc. Natl. Acad. Sci. USA 110 (31) (2013) 12601–12606, https://doi.org/10.1073/pnas.1306562110.
[27] R.A. Wimmer, A. Leopoldi, M. Aichinger, N. Wick, B. Hantusch, M. Novatchkova, J. Taubenschmid, M. Hämmerle, C. Esk, J.A. Bagley, D. Lindenhofer, G.B. Chen, M. Boehm, C.A. Agu, F.T. Yang, B.Y. Fu, J. Zuber, J.A. Knoblich, D. Kerjaschki, J. M. Penninger, Human blood vessel organoids as a model of diabetic vasculopathy, Nature 565 (7740) (2019) 505, https://doi.org/10.1038/s41586-018-0858-8.
[28] R.A. Wimmer, A. Leopoldi, M. Aichinger, D. Kerjaschki, J.M. Penninger, Generation of blood vessel organoids from human pluripotent stem cells, Nat. Protoc. 14 (11) (2019) 3082–3100, https://doi.org/10.1038/s41596-019-0213-z.
[29] A. Dailamy, U. Parekh, D. Katrekar, A. Kumar, D. McDonald, A. Moreno, P. Bagheri, T.N. Ng, P. Mali, Programmatic introduction of parenchymal cell types into blood vessel organoids, Stem Cell Rep. 16 (10) (2021) 2432–2441, https://doi.org/10.1016/j.stemcr.2021.08.014.
[30] S. Schmidt, Y. Alt, N. Deoghare, S. Krüger, A. Kern, A.F. Rockel, N. Wagner, S. Ergün, P. Wörsdörfer, A blood vessel organoid model recapitulating aspects of vasculogenesis, angiogenesis and vessel wall maturation, Organoids 1 (1) (2022) 41–53, https://doi.org/10.3390/organoids1010005.
[31] I. Salmon, S. Grebenyuk, A.R. Abdel Fattah, G. Rustandi, T. Pilkington, C. Verfaillie, A. Ranga, Engineering neurovascular organoids with 3D printed microfluidic chips, Lab Chip 22 (8) (2022) 1615–1629, https://doi.org/10.1039/d1lc00535a.
[32] Z. Ma, J. Wang, P. Loskill, N. Huebsch, S. Koo, F.L. Svedlund, N.C. Marks, E. W. Hua, C.P. Grigoropoulos, B.R. Conklin, K.E. Healy, Self-organizing human cardiac microchambers mediated by geometric confinement, Nat. Commun. 6 (1) (2015), https://doi.org/10.1038/ncomms8413.
[33] H.K. Voges, R.J. Mills, D.A. Elliott, R.G. Parton, E.R. Porrello, J.E. Hudson, Development of a human cardiac organoid injury model reveals innate regenerative potential, Development (Cambridge, England) 144 (6) (2017) 1118–1127, https://doi.org/10.1242/dev.143966.
[34] R.J. Mills, D.M. Titmarsh, X. Koenig, B.L. Parker, J.G. Ryall, G.A. Quaife-Ryan, H. K. Voges, M.P. Hodson, C. Ferguson, L. Drowley, A.T. Plowright, E.J. Needham, Q.-D. Wang, P. Gregorevic, M. Xin, W.G. Thomas, R.G. Parton, L.K. Nielsen, B. S. Launikonis, D.E. James, D.A. Elliott, E.R. Porrello, J.E. Hudson, Functional screening in human cardiac organoids reveals a metabolic mechanism for cardiomyocyte cell cycle arrest, Proc. Natl. Acad. Sci. USA 114 (40) (2017), https://doi.org/10.1073/pnas.1707316114.
[35] P. Hoang, J. Wang, B.R. Conklin, K.E. Healy, Z. Ma, Generation of spatial-patterned early-developing cardiac organoids using human pluripotent stem cells, Nat. Protoc. 13 (4) (2018) 723–737, https://doi.org/10.1038/nprot.2018.006.
[36] L. Drakhlis, S. Biswanath, C.M. Farr, V. Lupanow, J. Teske, K. Ritzenhoff, A. Franke, F. Manstein, E. Bolesani, H. Kempf, S. Liebscher, K. Schenke-Layland, J. Hegermann, L. Nolte, H. Meyer, J. de la Roche, S. Thiemann, C. Wahl-Schott, U. Martin, R. Zweigerdt, Human heart-forming organoids recapitulate early heart and foregut development, Nat. Biotechnol. 39 (6) (2021) 737–746, https://doi.org/10.1038/s41587-021-00815-9.
[37] S.G. Lee, Y.J. Kim, M.Y. Son, M.S. Oh, J. Kim, B. Ryu, K.R. Kang, J. Baek, G. Chung, D.H. Woo, C.Y. Kim, H.M. Chung, Generation of human iPSCs derived heart organoids structurally and functionally similar to heart, Biomaterials 290 (2022) 121860, https://doi.org/10.1016/j.biomaterials.2022.121860.
[38] B.X. Ho, J.K.S. Pang, Y. Chen, Y.H. Loh, O. An, H.H. Yang, V.P. Seshachalam, J.L. Y. Koh, W.K. Chan, S.Y. Ng, B.S. Soh, Robust generation of human-chambered cardiac organoids from pluripotent stem cells for improved modelling of cardiovascular diseases, Stem Cell Res. Ther. 13 (1) (2022) 529, https://doi.org/10.1186/s13287-022-03215-1.
[39] C. Schmidt, A. Deyett, T. Ilmer, S. Haendeler, A. Torres Caballero, M. Novatchkova, M.A. Netzer, L. Ceci Ginistrelli, E. Mancheno Juncosa, T. Bhattacharya, A. Mujadzic, L. Pimpale, S.M. Jahnel, M. Cirigliano, D. Reumann, K. Tavernini, N. Papai, S. Hering, P. Hofbauer, S. Mendjan, Multi-chamber cardioids unravel human heart development and cardiac defects, Cell 186 (25) (2023) 5587–5605.e27, https://doi.org/10.1016/j.cell.2023.10.030.
[40] P. Hofbauer, S.M. Jahnel, N. Papai, M. Giesshammer, A. Deyett, C. Schmidt, M. Penc, K. Tavernini, N. Grdseloff, C. Meledeth, L.C. Ginistrelli, C. Ctortecka, Š. Šalic, M. Novatchkova, S. Mendjan, Cardioids reveal self-organizing principles of human cardiogenesis, Cell 184 (12) (2021) 3299–3317.e22, https://doi.org/10.1016/j.cell.2021.04.034.
[41] B. Volmert, A. Kiselev, A. Juhong, F. Wang, A. Riggs, A. Kostina, C. O'Hern, P. Muniyandi, A. Wasserman, A. Huang, Y. Lewis-Israeli, V. Panda, S. Bhattacharya, A. Lauver, S. Park, Z. Qiu, C. Zhou, A. Aguirre, A patterned human primitive heart organoid model generated by pluripotent stem cell self-organization, Nat. Commun. 14 (1) (2023) 8245, https://doi.org/10.1038/s41467-023-43999-1.
[42] G. Rossi, N. Broguiere, M. Miyamoto, A. Boni, R. Guiet, M. Girgin, R.G. Kelly, C. Kwon, M.P. Lutolf, Capturing cardiogenesis in gastruloids, Cell Stem Cell 28 (2) (2021) 230–240.e6, https://doi.org/10.1016/j.stem.2020.10.013.
[43] W. Xuan, S.M. Tipparaju, M. Ashraf, Transformational applications of human cardiac organoids in cardiovascular diseases, Front. Cell Dev. Biol. 10 (2022) 936084, https://doi.org/10.3389/fcell.2022.936084.
[44] R.J. Mills, S.J. Humphrey, P.R.J. Fortuna, M. Lor, S.R. Foster, G.A. Quaife-Ryan, R.L. Johnston, T. Dumenil, C. Bishop, R. Rudraraju, D.J. Rawle, T. Le, W. Zhao, L. Lee, C. Mackenzie-Kludas, N.R. Mehdiabadi, C. Halliday, D. Gilham, L. Fu, S. J. Nicholls, J. Johansson, M. Sweeney, N.C.W. Wong, E. Kulikowski, K. A. Sokolowski, B.W.C. Tse, L. Devilée, H.K. Voges, L.T. Reynolds, S. Krumeich, E. Mathieson, D. Abu-Bonsrah, K. Karavendzas, B. Griffen, D. Titmarsh, D. A. Elliott, J. McMahon, A. Suhrbier, K. Subbarao, E.R. Porrello, M.J. Smyth, C. R. Engwerda, K.P.A. MacDonald, T. Bald, D.E. James, J.E. Hudson, BET inhibition blocks inflammation-induced cardiac dysfunction and SARS-CoV-2 infection, Cell 184 (8) (2021) 2167–2182.e22, https://doi.org/10.1016/j.cell.2021.03.026.
[45] K. Ronaldson-Bouchard, S.P. Ma, K. Yeager, T. Chen, L. Song, D. Sirabella, K. Morikawa, D. Teles, M. Yazawa, G. Vunjak-Novakovic, Advanced maturation of human cardiac tissue grown from pluripotent stem cells, Nature 556 (7700) (2018) 239–243, https://doi.org/10.1038/s41586-018-0016-3.
[46] Y.R. Lewis-Israeli, B.D. Volmert, M.A. Gabalski, A.R. Huang, A. Aguirre, Generating self-assembling human heart organoids derived from pluripotent stem cells, Jove-J Vis Exp 175 (2021). ARTNe6309710.3791/63097.
[47] M. Prondzynski, P. Berkson, M.A. Trembley, Y. Tharani, K. Shani, R.H. Bortolin, M.E. Sweat, J. Mayourian, D. Yucel, A.M. Cordoves, B. Gabbin, C. Hou, N. J. Anyanwu, F. Nawar, J. Cotton, J. Milosh, D. Walker, Y. Zhang, F. Lu, X. Liu, K. K. Parker, V.J. Bezzerides, W.T. Pu, Efficient and reproducible generation of human iPSC-derived cardiomyocytes and cardiac organoids in stirred suspension systems, Nat. Commun. 15 (1) (2024), https://doi.org/10.1038/s41467-024-50224-0.
[48] S.G. Romeo, I. Secco, E. Schneider, C.M. Reumiller, C.X.C. Santos, A. Zoccarato, V. Musale, A. Pooni, X. Yin, K. Theofilatos, S.C. Trevelin, L. Zeng, G.E. Mann, V. Pathak, K. Harkin, A.W. Stitt, R.J. Medina, A. Margariti, M. Mayr, A.M. Shah, M. Giacca, A. Zampetaki, Human blood vessel organoids reveal a critical role for CTGF in maintaining microvascular integrity, Nat. Commun. 14 (1) (2023) 5552, https://doi.org/10.1038/s41467-023-41326-2.
[49] E.M. Meijer, S.E. Koch, C.G.M. van Dijk, R.G.C. Maas, I. Chrifi, W. Szymczyk, P. J. Besseling, L. Pomp, V.J.C.H. Koomen, J.W. Buikema, C.V.C. Bouten, M. C. Verhaar, A.I.P.M. Smits, C. Cheng, 3D human iPSC blood vessel organoids as a source of flow-adaptive vascular cells for creating a human-relevant 3D-scaffold based macrovessel model, Advanced Biology 7 (1) (2023), https://doi.org/10.1002/adbi.202200137.
[50] S.V. Murphy, A. Atala, 3D bioprinting of tissues and organs, Nat. Biotechnol. 32 (8) (2014) 773–785, https://doi.org/10.1038/nbt.2958.
[51] J. Malda, J. Visser, F.P. Melchels, T. Jüngst, W.E. Hennink, W.J.A. Dhert, J. Groll, D.W. Hutmacher, 25th anniversary article: engineering hydrogels for biofabrication, Adv. Mater. 25 (36) (2013) 5011–5028, https://doi.org/10.1002/adma.201302042.
[52] D.B. Kolesky, R.L. Truby, A.S. Gladman, T.A. Busbee, K.A. Homan, J.A. Lewis, 3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs, Adv. Mater. 26 (19) (2014) 3124–3130, https://doi.org/10.1002/adma.201305506.
[53] N. Noor, A. Shapira, R. Edri, I. Gal, L. Wertheim, T. Dvir, 3D printing of personalized thick and perfusable cardiac patches and hearts, Adv. Sci. 6 (11) (2019) 1900344, https://doi.org/10.1002/advs.201900344.
[54] M. Alonzo, R. El Khoury, N. Nagiah, V. Thakur, M. Chattopadhyay, B. Joddar, 3D biofabrication of a cardiac tissue construct for sustained longevity and function, ACS Appl. Mater. Interfaces 14 (19) (2022) 21800–21813, https://doi.org/10.1021/acsami.1c23883.
[55] Y.S. Zhang, A. Arneri, S. Bersini, S.-R. Shin, K. Zhu, Z. Goli-Malekabadi, J. Aleman, C. Colosi, F. Busignani, V. Dell'Erba, C. Bishop, T. Shupe, D. Demarchi, M. Moretti, M. Rasponi, M.R. Dokmeci, A. Atala, A. Khademhosseini, Bioprinting 3D microfibrous scaffolds for engineering endothelialized myocardium and heart-on-a-chip, Biomaterials 110 (2016) 45–59, https://doi.org/10.1016/j.biomaterials.2016.09.003.
[56] F. Maiullari, M. Costantini, M. Milan, V. Pace, M. Chirivì, S. Maiullari, A. Rainer, D. Baci, H.E.-S. Marei, D. Seliktar, C. Gargioli, C. Bearzi, R. Rizzi, A multi-cellular 3D bioprinting approach for vascularized heart tissue engineering based on HUVECs and iPSC-derived cardiomyocytes, Sci. Rep. 8 (1) (2018), https://doi.org/10.1038/s41598-018-31848-x.
[57] E.K. Sackmann, A.L. Fulton, D.J. Beebe, The present and future role of microfluidics in biomedical research, Nature 507 (7491) (2014) 181–189, https://doi.org/10.1038/nature13118.
[58] W. Zheng, B. Jiang, D. Wang, W. Zhang, Z. Wang, X. Jiang, A microfluidic flow-stretch chip for investigating blood vessel biomechanics, Lab Chip 12 (18) (2012), https://doi.org/10.1039/c2lc40173h.
[59] X. Wang, D.T.T. Phan, A. Sobrino, S.C. George, C.C.W. Hughes, A.P. Lee, Engineering anastomosis between living capillary networks and endothelial cell-lined microfluidic channels, Lab Chip 16 (2) (2016) 282–290, https://doi.org/10.1039/c5lc01050k.
[60] C. Quintard, E. Tubbs, G. Jonsson, J. Jiao, J. Wang, N. Werschler, C. Laporte, A. Pitaval, T.S. Bah, G. Pomeranz, C. Bissardon, J. Kaal, A. Leopoldi, D.A. Long, P. Blandin, J.L. Achard, C. Battail, A. Hagelkruys, F. Navarro, Y. Fouillet, J. M. Penninger, X. Gidrol, A microfluidic platform integrating functional vascularized organoids-on-chip, Nat. Commun. 15 (1) (2024). ARTN145210.1038/s41467-024-45710-4.
[61] A. Khademhosseini, G. Eng, J. Yeh, P.A. Kucharczyk, R. Langer, G. Vunjak-Novakovic, M. Radisic, Microfluidic patterning for fabrication of contractile cardiac organoids, Biomed. Microdevices 9 (2) (2006) 149–157, https://doi.org/10.1007/s10544-006-9013-7.
[62] A. Marsano, C. Conficconi, M. Lemme, P. Occhetta, E. Gaudiello, E. Votta, G. Cerino, A. Redaelli, M. Rasponi, Beating heart on a chip: a novel microfluidic platform to generate functional 3D cardiac microtissues, Lab Chip 16 (3) (2016) 599–610, https://doi.org/10.1039/c5lc01356a.
[63] K. Moshksayan, A. Harihara, S. Mondal, E. Hegarty, T. Atherly, D.K. Sahoo, A. E. Jergens, J.P. Mochel, K. Allenspach, J. Zoldan, A. Ben-Yakar, OrganoidChip facilitates hydrogel-free immobilization for fast and blur-free imaging of organoids, Sci. Rep. 13 (1) (2023), https://doi.org/10.1038/s41598-023-38212-8.
[64] F.Y. Li, X. Gou, D. Xu, D. Han, K. Hou, W.R. Fang, Y.M. Li, Improvement of tube formation model of cell: application for acute hypoxia in in vitro study of

angiogenesis, Microvasc. Res. 140 (2022). ARTN10429710.1016/j.mvr.2021.104297.
[65] N. Werschler, J. Penninger, Generation of human blood vessel organoids from pluripotent stem cells, Jove-J Vis Exp 191 (2023), https://doi.org/10.3791/64715.
[66] D.J. Richards, Y. Li, C.M. Kerr, J. Yao, G.C. Beeson, R.C. Coyle, X. Chen, J. Jia, B. Damon, R. Wilson, E. Starr Hazard, G. Hardiman, D.R. Menick, C.C. Beeson, H. Yao, T. Ye, Y. Mei, Human cardiac organoids for the modelling of myocardial infarction and drug cardiotoxicity, Nat. Biomed. Eng. 4 (4) (2020) 446–462, https://doi.org/10.1038/s41551-020-0539-4.
[67] Y.R. Lewis-Israeli, M. Abdelhamid, I. Olomu, A. Aguirre, Modeling the effects of maternal diabetes on the developing human heart using pluripotent stem cell–derived heart organoids, Current Protocols 2 (6) (2022), https://doi.org/10.1002/cpz1.461.
[68] R. Shinnawi, N. Shaheen, I. Huber, A. Shiti, G. Arbel, A. Gepstein, N. Ballan, N. Setter, A.J. Tijsen, M. Borggrefe, L. Gepstein, Modeling reentry in the short QT syndrome with human-induced pluripotent stem cell-derived cardiac cell sheets, J. Am. Coll. Cardiol. 73 (18) (2019) 2310–2324, https://doi.org/10.1016/j.jacc.2019.02.055.
[69] M. Tiburcy, J.E. Hudson, P. Balfanz, S. Schlick, T. Meyer, M.L. Chang Liao, E. Levent, F. Raad, S. Zeidler, E. Wingender, J. Riegler, M. Wang, J.D. Gold, I. Kehat, E. Wettwer, U. Ravens, P. Dierickx, L.W. van Laake, M.J. Goumans, S. Khadjeh, K. Toischer, G. Hasenfuss, L.A. Couture, A. Unger, W.A. Linke, T. Araki, B. Neel, G. Keller, L. Gepstein, J.C. Wu, W.H. Zimmermann, Defined engineered human myocardium with advanced maturation for applications in heart failure modeling and repair, Circulation 135 (19) (2017) 1832–1847, https://doi.org/10.1161/circulationaha.116.024145.
[70] W. Wang, J. Yang, P. Kang, J. Bai, X. Feng, L. Huang, Y. Zhang, Y. Wu, B. Tang, H. Wang, J. Jiang, M. Li, B. Zhao, X. Yang, Direct infection of SARS-CoV-2 in human iPSC-derived 3D cardiac organoids recapitulates COVID-19 myocarditis, Virol. Sin. 38 (6) (2023) 971–974, https://doi.org/10.1016/j.virs.2023.09.005.
[71] A. Kostina, Y.R. Lewis-Israeli, M. Abdelhamid, M.A. Gabalski, A. Kiselev, B. D. Volmert, H. Lankerd, A.R. Huang, A.H. Wasserman, T. Lydic, C. Chan, S. Park, I. Olomu, A. Aguirre, ER stress and lipid imbalance drive diabetic embryonic cardiomyopathy in an organoid model of human heart development, Stem Cell Rep. 19 (3) (2024) 317–330, https://doi.org/10.1016/j.stemcr.2024.01.003.
[72] N. Huebsch, P. Loskill, M.A. Mandegar, N.C. Marks, A.S. Sheehan, Z. Ma, A. Mathur, T.N. Nguyen, J.C. Yoo, L.M. Judge, C.I. Spencer, A.C. Chukka, C. R. Russell, P.L. So, B.R. Conklin, K.E. Healy, Automated video-based analysis of contractility and calcium flux in human-induced pluripotent stem cell-derived cardiomyocytes cultured over different spatial scales, Tissue Eng Part C-Me 21 (5) (2015) 467–479, https://doi.org/10.1089/ten.tec.2014.0283.
[73] Y. Ming, S. Hao, F. Wang, Y.R. Lewis-Israeli, B.D. Volmert, Z. Xu, A. Goestenkors, A. Aguirre, C. Zhou, Longitudinal morphological and functional characterization of human heart organoids using optical coherence tomography, Biosens. Bioelectron. 207 (2022), https://doi.org/10.1016/j.bios.2022.114136.
[74] E.G. Navarrete, P. Liang, F. Lan, V. Sanchez-Freire, C. Simmons, T. Gong, A. Sharma, P.W. Burridge, B. Patlolla, A.S. Lee, H. Wu, R.E. Beygui, S.M. Wu, R. C. Robbins, D.M. Bers, J.C. Wu, Screening drug-induced arrhythmia using human induced pluripotent stem cell–derived cardiomyocytes and low-impedance microelectrode arrays, Circulation 128 (11_suppl_1) (2013), https://doi.org/10.1161/circulationaha.112.000570.
[75] A.P. Passaro, S.L. Stice, Electrophysiological analysis of brain organoids: current approaches and advancements, Front. Neurosci. 14 (2021). ARTN62213710.3389/fnins.2020.622137.
[76] Y. Yamamoto, S. Hirose, Y. Wuriyanghai, D. Yoshinaga, T. Makiyama, Electrophysiological Analysis of hiPSC-Derived Cardiomyocytes Using a Patch-Clamp Technique, Pluripotent Stem-Cell Derived Cardiomyocytes2021, pp. 121-133. https://doi.org/10.1007/978-1-0716-1484-6_13.
[77] J. Gao, C. Liao, S. Liu, T. Xia, G. Jiang, Nanotechnology: new opportunities for the development of patch-clamps, J. Nanobiotechnol. 19 (1) (2021), https://doi.org/10.1186/s12951-021-00841-4.
[78] F.L. Lampart, D. Iber, N. Doumpas, Organoids in high-throughput and high-content screenings, Front Chem Eng 5 (2023). ARTN112034810.3389/fceng.2023.1120348.
[79] Y.C. Wang, H. Jeon, 3D cell cultures toward quantitative high-throughput drug screening, Trends Pharmacol. Sci. 43 (7) (2022) 569–581, https://doi.org/10.1016/j.tips.2022.03.014569.
[80] N. Brandenberg, S. Hoehnel, F. Kuttler, K. Homicsko, C. Ceroni, T. Ringel, N. Gjorevski, G. Schwank, G. Coukos, G. Turcatti, M.P. Lutolf, High-throughput automated organoid culture via stem-cell aggregation in microcavity arrays, Nat. Biomed. Eng. 4 (9) (2020) 863–874, https://doi.org/10.1038/s41551-020-0565-2.
[81] A. Louey, D. Hernández, A. Pébay, M. Daniszewski, Automation of organoid cultures: current protocols and applications, SLAS Discovery 26 (9) (2021) 1138–1147, https://doi.org/10.1177/24725552211024547.
[82] D. Schneidman-Duhovny, J.M. Matthews, B. Schuster, S.S. Kashaf, P. Liu, R. Ben-Yishay, D. Ishay-Ronen, E. Izumchenko, L. Shen, C.R. Weber, M. Bielski, S. S. Kupfer, M. Bilgic, A. Rzhetsky, S. Tay, OrganoID: a versatile deep learning platform for tracking and analysis of single-organoid dynamics, PLoS Comput. Biol. 18 (11) (2022), https://doi.org/10.1371/journal.pcbi.1010584.
[83] A. Kostina, B. Volmert, A. Aguirre, Human heart organoids: current applications and future perspectives, Eur. Heart J. 45 (10) (2024) 751–753, https://doi.org/10.1093/eurheartj/ehad841.
[84] M. Sahara, Recent advances in generation of in vitro cardiac organoids, Int. J. Mol. Sci. 24 (7) (2023), https://doi.org/10.3390/ijms24076244.
[85] H.K. Voges, S.R. Foster, L. Reynolds, B.L. Parker, L. Devilée, G.A. Quaife-Ryan, P. R.J. Fortuna, E. Mathieson, R. Fitzsimmons, M. Lor, C. Batho, J. Reid, M. Pocock, C.E. Friedman, D. Mizikovsky, M. Francois, N.J. Palpant, E.J. Needham, M. Peralta, G.D. Monte-Nieto, L.K. Jones, I.M. Smyth, N.R. Mehdiabadi, F. Bolk, V. Janbandhu, E. Yao, R.P. Harvey, J.J.H. Chong, D.A. Elliott, E.G. Stanley, S. Wiszniak, Q. Schwarz, D.E. James, R.J. Mills, E.R. Porrello, J.E. Hudson, Vascular cells improve functionality of human cardiac organoids, Cell Rep. 42 (5) (2023) 112322, https://doi.org/10.1016/j.celrep.2023.112322.
[86] J. He, D. Liu, L. Zhao, D. Zhou, J. Rong, L. Zhang, Z. Xia, Myocardial ischemia/reperfusion injury: Mechanisms of injury and implications for management (Review), Exp. Ther. Med. 23 (6) (2022) 430, 10.3892/etm.2022.11357.
[87] M. Hesse, B.K. Fleischmann, M.I. Kotlikoff, Concise review: the role of C-kit expressing cells in heart repair at the neonatal and adult stage, Stem. Cells 32 (7) (2014) 1701–1712, https://doi.org/10.1002/stem.1696.
[88] T.J. Cahill, H. Ashrafian, H. Watkins, Genetic cardiomyopathies causing heart failure, Circ. Res. 113 (6) (2013) 660–675, https://doi.org/10.1161/circresaha.113.300282.
[89] J.B. Cole, J.C. Florez, Genetics of diabetes mellitus and diabetes complications, Nat. Rev. Nephrol. 16 (7) (2020) 377–390, https://doi.org/10.1038/s41581-020-0278-5.
[90] R. Morizane, Modelling diabetic vasculopathy with human vessel organoids, Nat. Rev. Nephrol. 15 (5) (2019) 258–260, https://doi.org/10.1038/s41581-019-0125-8.
[91] O. Mourad, R. Yee, M. Li, S.S. Nunes, Modeling heart diseases on a chip: advantages and future opportunities, Circ. Res. 132 (4) (2023) 483–497, https://doi.org/10.1161/circresaha.122.321670.
[92] M. Prondzynski, M.D. Lemoine, A.T. Zech, A. Horváth, V. Di Mauro, J. T. Koivumäki, N. Kresin, J. Busch, T. Krause, E. Krämer, S. Schlossarek, M. Spohn, F.W. Friedrich, J. Münch, S.D. Laufer, C. Redwood, A.E. Volk, A. Hansen, G. Mearini, D. Catalucci, C. Meyer, T. Christ, M. Patten, T. Eschenhagen, L. Carrier, Disease modeling of a mutation in α-actinin 2 guides clinical therapy in hypertrophic cardiomyopathy, EMBO Mol. Med. 11 (12) (2019) e11115, https://doi.org/10.15252/emmm.201911115.
[93] M. Filippo Buono, L. von Boehmer, J. Strang, S.P. Hoerstrup, M.Y. Emmert, B. Nugraha, Human cardiac organoids for modeling genetic cardiomyopathy, Cells 9 (7) (2020), https://doi.org/10.3390/cells9071733.
[94] V. Marini, F. Marino, F. Aliberti, N. Giarratana, E. Pozzo, R. Duelen, Á. Cortés Calabuig, R. La Rovere, T. Vervliet, D. Torella, G. Bultynck, M. Sampaolesi, Y. C. Chai, Long-term culture of patient-derived cardiac organoids recapitulated Duchenne muscular dystrophy cardiomyopathy and disease progression, Front. Cell Dev. Biol. 10 (2022) 878311, https://doi.org/10.3389/fcell.2022.878311.
[95] F. Omidi, B. Hajikhani, S.N. Kazemi, A. Tajbakhsh, S. Riazi, M. Mirsaeidi, A. Ansari, M. Ghanbari Boroujeni, F. Khalili, S. Hadadi, M.J. Nasiri, COVID-19 and cardiomyopathy: a systematic review, Front. Cardiovasc. Med 8 (2021) 695206, https://doi.org/10.3389/fcvm.2021.695206.
[96] C.M. Wolf, C.I. Berul, Inherited conduction system abnormalities–one group of diseases, many genes, J. Cardiovasc. Electrophysiol. 17 (4) (2006) 446–455, https://doi.org/10.1111/j.1540-8167.2006.00427.x.
[97] J.S. Hulot, Modeling cardiac arrhythmias with organoids, J. Am. Coll. Cardiol. 73 (18) (2019) 2325–2327, https://doi.org/10.1016/j.jacc.2019.01.076.
[98] I. El-Battrawy, H. Lan, L. Cyganek, Z. Zhao, X. Li, F. Buljubasic, S. Lang, G. Yücel, K. Sattler, W.H. Zimmermann, J. Utikal, T. Wieland, U. Ravens, M. Borggrefe, X. B. Zhou, I. Akin, Modeling short QT syndrome using human-induced pluripotent stem cell-derived cardiomyocytes, J. Am. Heart Assoc. 7 (7) (2018), https://doi.org/10.1161/jaha.117.007394.
[99] M. Ghosheh, A. Ehrlich, K. Ioannidis, M. Ayyash, I. Goldfracht, M. Cohen, A. Fischer, Y. Mintz, L. Gepstein, Y. Nahmias, Electro-metabolic coupling in multi-chambered vascularized human cardiac organoids, Nat. Biomed. Eng. 7 (11) (2023) 1493–1513, https://doi.org/10.1038/s41551-023-01071-9.
[100] L.E. Mokry, O. Ahmad, V. Forgetta, G. Thanassoulis, J.B. Richards, Mendelian randomisation applied to drug development in cardiovascular disease: a review, J. Med. Genet. 52 (2) (2015) 71–79, https://doi.org/10.1136/jmedgenet-2014-102438.
[101] R.J. Mills, B.L. Parker, G.A. Quaife-Ryan, H.K. Voges, E.J. Needham, A. Bornot, M. Ding, H. Andersson, M. Polla, D.A. Elliott, L. Drowley, M. Clausen, A. T. Plowright, I.P. Barrett, Q.D. Wang, D.E. James, E.R. Porrello, J.E. Hudson, Drug screening in human PSC-cardiac organoids identifies pro-proliferative compounds acting via the mevalonate pathway, Cell Stem Cell 24 (6) (2019) 895–907.e6, https://doi.org/10.1016/j.stem.2019.03.009.
[102] T. Kitsuka, M. Itoh, S. Amamoto, K.I. Arai, J. Oyama, K. Node, S. Toda, S. Morita, T. Nishida, K. Nakayama, 2-Cl-C.OXT-A stimulates contraction through the suppression of phosphodiesterase activity in human induced pluripotent stem cell-derived cardiac organoids, PLoS One 14 (7) (2019) e0213114, https://doi.org/10.1371/journal.pone.0213114.
[103] P. Hoang, A. Kowalczewski, S.Y. Sun, T.S. Winston, A.M. Archilla, S.M. Lemus, A. G. Ercan-Sencicek, A.R. Gupta, W.Z. Liu, M.I. Kontaridis, J.D. Amack, Z. Ma, Engineering spatial-organized cardiac organoids for developmental toxicity testing, Stem Cell Rep. 16 (5) (2021) 1228–1244, https://doi.org/10.1016/j.stemcr.2021.03.013.
[104] D. Fatkin, I.G. Huttner, J.C. Kovacic, J.G. Seidman, C.E. Seidman, Precision medicine in the management of dilated cardiomyopathy: JACC state-of-the-art review, J. Am. Coll. Cardiol. 74 (23) (2019) 2921–2938, https://doi.org/10.1016/j.jacc.2019.10.011.
[105] K. Musunuru, F. Sheikh, R.M. Gupta, S.R. Houser, K.O. Maher, D.J. Milan, A. Terzic, J.C. Wu, Induced pluripotent stem cells for cardiovascular disease modeling and precision medicine: a scientific statement from the American heart

association, circ. Genom. Precis, Med 11 (1) (2018) e000043, https://doi.org/10.1161/hcg.0000000000000043.
[106] D.T. Paik, M. Chandy, J.C. Wu, Patient and disease-specific induced pluripotent stem cells for discovery of personalized cardiovascular drugs and therapeutics, Pharmacol. Rev. 72 (1) (2020) 320–342, https://doi.org/10.1124/pr.116.013003.
[107] O. Bergmann, R.D. Bhardwaj, S. Bernard, S. Zdunek, F. Barnabé-Heider, S. Walsh, J. Zupicich, K. Alkass, B.A. Buchholz, H. Druid, S. Jovinge, J. Frisén, Evidence for cardiomyocyte renewal in humans, Science 324 (5923) (2009) 98–102, https://doi.org/10.1126/science.1164680.
[108] Y. Tian, Y. Liu, T. Wang, N. Zhou, J. Kong, L. Chen, M. Snitow, M. Morley, D. Li, N. Petrenko, S. Zhou, M. Lu, E. Gao, W.J. Koch, K.M. Stewart, E.E. Morrisey, A microRNA-Hippo pathway that promotes cardiomyocyte proliferation and cardiac regeneration in mice, Sci. Transl. Med. 7 (279) (2015) 279ra38, https://doi.org/10.1126/scitranslmed.3010841.
[109] S. Hashmi, H.R. Ahmad, Molecular switch model for cardiomyocyte proliferation, Cell Regen. 8 (1) (2019) 12–20, https://doi.org/10.1016/j.cr.2018.11.002.
[110] M. Mazzola, E. Di Pasquale, Toward cardiac regeneration: combination of pluripotent stem cell-based therapies and bioengineering strategies, Front. Bioeng. Biotechnol. 8 (2020) 455, https://doi.org/10.3389/fbioe.2020.00455.
[111] F. Varzideh, S. Pahlavan, H. Ansari, M. Halvaei, S. Kostin, M.S. Feiz, H. Latifi, N. Aghdami, T. Braun, H. Baharvand, Human cardiomyocytes undergo enhanced maturation in embryonic stem cell-derived organoid transplants, Biomaterials 192 (2019) 537–550, https://doi.org/10.1016/j.biomaterials.2018.11.033.
[112] X. Dong, S.-B. Xu, X. Chen, M. Tao, X.-Y. Tang, K.-H. Fang, M. Xu, Y. Pan, Y. Chen, S. He, Y. Liu, Human cerebral organoids establish subcortical projections in the mouse brain after transplantation, Mol. Psychiatry 26 (7) (2020) 2964–2976, https://doi.org/10.1038/s41380-020-00910-4.
[113] O. Revah, F. Gore, K.W. Kelley, J. Andersen, N. Sakai, X. Chen, M.-Y. Li, F. Birey, X. Yang, N.L. Saw, S.W. Baker, N.D. Amin, S. Kulkarni, R. Mudipalli, B. Cui, S. Nishino, G.A. Grant, J.K. Knowles, M. Shamloo, J.R. Huguenard, K. Deisseroth, S.P. Pașca, Maturation and circuit integration of transplanted human cortical organoids, Nature 610 (7931) (2022) 319–326, https://doi.org/10.1038/s41586-022-05277-w.
[114] Y. Tan, R.C. Coyle, R.W. Barrs, S.E. Silver, M. Li, D.J. Richards, Y. Lin, Y. Jiang, H. Wang, D.R. Menick, K. Deleon-Pennell, B. Tian, Y. Mei, Nanowired human cardiac organoid transplantation enables highly efficient and effective recovery of infarcted hearts, Sci. Adv. 9 (31) (2023) eadf2898, https://doi.org/10.1126/sciadv.adf2898.
[115] S. Miyagawa, S. Kainuma, T. Kawamura, K. Suzuki, Y. Ito, H. Iseoka, E. Ito, M. Takeda, M. Sasai, N. Mochizuki-Oda, T. Shimamoto, Y. Nitta, H. Dohi, T. Watabe, Y. Sakata, K. Toda, Y. Sawa, Case report: transplantation of human induced pluripotent stem cell-derived cardiomyocyte patches for ischemic cardiomyopathy, Front. Cardiovasc. Med 9 (2022) 950829, https://doi.org/10.3389/fcvm.2022.950829.
[116] D. Chang, Z. Wen, Y. Wang, W. Cai, M. Wani, C. Paul, T. Okano, R.W. Millard, Y. Wang, Ultrastructural features of ischemic tissue following application of a bio-membrane based progenitor cardiomyocyte patch for myocardial infarction repair, PLoS One 9 (10) (2014) e107296, https://doi.org/10.1371/journal.pone.0107296.
[117] Y. Ahn, J.H. An, H.J. Yang, D.G. Lee, J. Kim, H. Koh, Y.H. Park, B.S. Song, B. W. Sim, H.J. Lee, J.H. Lee, S.U. Kim, Human blood vessel organoids penetrate human cerebral organoids and form a vessel-like system, Cells 10 (8) (2021), https://doi.org/10.3390/cells10082036.
[118] M.A. Lancaster, M. Huch, Disease modelling in human organoids, Dis. Model. Mech 12 (7) (2019), https://doi.org/10.1242/dmm.039347.
[119] V. Boyalla, E. Gallego-Colon, M. Spartalis, Immunity and inflammation in cardiovascular disorders, BMC Cardiovasc. Disord. 23 (1) (2023). ARTN14810.1186/s12872-023-03185-z.
[120] G. Quadrato, T. Nguyen, E.Z. Macosko, J.L. Sherwood, S. Min Yang, D.R. Berger, N. Maria, J. Scholvin, M. Goldman, J.P. Kinney, E.S. Boyden, J.W. Lichtman, Z. M. Williams, S.A. McCarroll, P. Arlotta, Cell diversity and network dynamics in photosensitive human brain organoids, Nature 545 (7652) (2017) 48–53, https://doi.org/10.1038/nature22047.
[121] M.E. Kupfer, W.-H. Lin, V. Ravikumar, K. Qiu, L. Wang, L. Gao, D.B. Bhuiyan, M. Lenz, J. Ai, R.R. Mahutga, D. Townsend, J. Zhang, M.C. McAlpine, E. G. Tolkacheva, B.M. Ogle, In situ expansion, differentiation, and electromechanical coupling of human cardiac muscle in a 3D bioprinted, chambered organoid, Circ. Res. 127 (2) (2020) 207–224, https://doi.org/10.1161/circresaha.119.316155.
[122] H. Liu, Z. Gan, X. Qin, Y. Wang, J. Qin, Advances in microfluidic technologies in organoid research, Adv. Healthcare Mater. 13 (21) (2023), https://doi.org/10.1002/adhm.202302686.
[123] H. Wang, X. Ning, F. Zhao, H. Zhao, D. Li, Human organoids-on-chips for biomedical research and applications, Theranostics 14 (2) (2024) 788–818, https://doi.org/10.7150/thno.90492.
[124] C.S. Ong, L. Nam, K. Ong, A. Krishnan, C.Y. Huang, T. Fukunishi, N. Hibino, 3D and 4D bioprinting of the myocardium: current approaches, challenges, and future prospects, BioMed Res. Int. 2018 (2018) 1–11, https://doi.org/10.1155/2018/6497242.
[125] B. Nugraha, M.F. Buono, L. von Boehmer, S.P. Hoerstrup, M.Y. Emmert, Human cardiac organoids for disease modeling, Clin. Pharmacol. Ther 105 (1) (2019) 79–85, https://doi.org/10.1002/cpt.1286.
[126] M. Martin, E.K.N. Gähwiler, M. Generali, S.P. Hoerstrup, M.Y. Emmert, Advances in 3D organoid models for stem cell-based cardiac regeneration, Int. J. Mol. Sci. 24 (6) (2023), https://doi.org/10.3390/ijms24065188.
[127] R. Lehmann, C.M. Lee, E.C. Shugart, M. Benedetti, R.A. Charo, Z. Gartner, B. Hogan, J. Knoblich, C.M. Nelson, K.M. Wilson, Human organoids: a new dimension in cell biology, Mol. Biol. Cell 30 (10) (2019) 1129–1137, https://doi.org/10.1091/mbc.E19-03-0135.
[128] X. Yin, B.E. Mead, H. Safaee, R. Langer, J.M. Karp, O. Levy, Engineering stem cell organoids, cell. Stem, Cell 18 (1) (2016) 25–38, https://doi.org/10.1016/j.stem.2015.12.005.
[129] S. Hao, C. Ren, F. Wang, K. Park, B.D. Volmert, A. Aguirre, C. Zhou, A Dual-modality imaging system for monitoring human heart organoids beating in vitro, Opt Lett. 48 (15) (2023), https://doi.org/10.1364/ol.493824.
[130] A.J. Deloria, S. Haider, B. Dietrich, V. Kunihs, S. Oberhofer, M. Knofler, R. Leitgeb, M.Y. Liu, W. Drexler, R. Haindl, Ultra-high-resolution 3D optical coherence tomography reveals inner structures of human placenta-derived trophoblast organoids, IEEE Trans. Biomed. Eng. 68 (8) (2021) 2368–2376, https://doi.org/10.1109/Tbme.2020.3038466.
[131] J.M. Bliley, M.A. Stang, A. Behre, A.W. Feinberg, Advances in 3D bioprinted cardiac tissue using stem cell-derived cardiomyocytes, Stem Cell Transl Med 13 (5) (2024) 425–435, https://doi.org/10.1093/stcltm/szae014.
[132] J. Shin, H. Chung, H. Kumar, K. Meadows, S. Park, J. Chun, K. Kim, 3D bioprinting of human iPSC-Derived kidney organoids using a low-cost, high-throughput customizable 3D bioprinting system, Bioprinting 38 (2024), https://doi.org/10.1016/j.bprint.2024.e00337.
[133] D. de Jongh, E.K. Massey, E.M. Bunnik, Organoids: a systematic review of ethical issues, Stem, Cell. Res. Ther 13 (1) (2022) 337, https://doi.org/10.1186/s13287-022-02950-9.
[134] M.A. Lensink, S.N. Boers, M.G. Va, K.R. Jongsma, A.L. Bredenoord, Mini-gut feelings: perspectives of people with cystic fibrosis on the ethics and governance of organoid biobanking, Per, Med 18 (3) (2021) 241–254, https://doi.org/10.2217/pme-2020-0161.
[135] A.L. Bredenoord, H. Clevers, J.A. Knoblich, Human tissues in a dish: the research and ethical implications of organoid technology, Science 355 (6322) (2017), https://doi.org/10.1126/science.aaf9414.
[136] M. Song, D.B. Choi, J.S. Im, Y.N. Song, J.H. Kim, H. Lee, J. An, A. Kim, H. Choi, J.-C. Kim, C. Han, Y.K. Jeon, S.J. Kim, D.-H. Woo, Modeling acute myocardial infarction and cardiac fibrosis using human induced pluripotent stem cell-derived multi-cellular heart organoids, Cell Death Dis. 15 (5) (2024), https://doi.org/10.1038/s41419-024-06703-9.
[137] B.M. Poh, L.C. Liew, Y.N.A. Soh, R.C. Lai, S.K. Lim, Y.S. Ho, B.S. Soh, MSC-derived small extracellular vesicles exert cardioprotective effect through reducing VLCFAs and apoptosis in human cardiac organoid IRI model, Stem cells (Dayton, Ohio) 42 (5) (2024) 416–429, https://doi.org/10.1093/stmcls/sxae015.
[138] K. Künzel, S.R. Künzel, K.M. Guan, Generation of self-assembling cardiac organoids using hiPSC-derived cardiomyocytes, Heliyon 8 (9) (2022) e10365. ARTNe1036510.1016/j.heliyon.2022.e10365.