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Review article

Progress of organoid platform in cardiovascular research

Xingchao Du [a,b], Hao Jia [a,b], Yuan Chang [a,b], Yiqi Zhao [a,b], Jiangping Song [a,b,*]

[a] Beijing Key Laboratory of Preclinical Research and Evaluation for Cardiovascular Implant Materials, Animal Experimental Centre, National Centre for Cardiovascular Disease, Department of Cardiac Surgery, Fuwai Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
[b] State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, Chinese Academy of Medical Science, PUMC, 167 Beilishi Road, Xicheng District, Beijing, 100037, China

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ABSTRACT

Cardiovascular disease is a significant cause of death in humans. Various models are necessary for the study of cardiovascular diseases, but once cellular and animal models have some defects, such as insufficient fidelity. As a new technology, organoid has certain advantages and has been used in many applications in the study of cardiovascular diseases. This article aims to summarize the application of organoid platforms in cardiovascular diseases, including organoid construction schemes, modeling, and application of cardiovascular organoids. Advances in cardiovascular organoid research have provided many models for different cardiovascular diseases in a variety of areas, including myocardium, blood vessels, and valves. Physiological and pathological models of different diseases, drug research models, and methods for evaluating and promoting the maturation of different kinds of organ tissues are provided for various cardiovascular diseases, including cardiomyopathy, myocardial infarction, and atherosclerosis. This article provides a comprehensive overview of the latest research progress in cardiovascular organ tissues, including construction protocols for cardiovascular organoid tissues and their evaluation system, different types of disease models, and applications of cardiovascular organoid models in various studies. The problems and possible solutions in organoid development are summarized.

1. Introduction

According to WHO 2020, ischaemic heart disease accounts for 16 % of all deaths worldwide [1]. Cardiovascular disease (CVD) accounted for approximately one-third of all deaths worldwide in 2019 [2]. Further conquering cardiovascular diseases has become an important aspect of solving health problems. As early as the early 20th century, research into cardiovascular disease began. Traditional heart models have been based primarily on two-dimensional (2D) cultures of cardiomyocytes (CMs), which can be derived from embryonic or newborn heart tissue or stem cells [3]. CMs can also differentiate from induced pluripotent stem cells (iPSCs): In 2006, research by Takahashi and Yamanaka demonstrated how ectopic co-expression of transcription factors alters cell fate [4]. Manipulating cell fate through reprogramming obtained iPSCs for disease research [5,6]. Animal models are also widely used in cardiovascular disease studies, such as a mouse model of chronic stress affecting the cardiovascular system [7]. It is recommended that studies be conducted on small mammalian models of ischemia-reperfusion injury (IRI) and heart failure (HF), such as mice and rabbits, and large non-human mammalian models, such as dogs, pigs, or primates [8]. Additionally, a mouse model of Crispr-Cas9 gene editing also appeared in 2013 [9]. Nevertheless, 2D models have many areas for improvement. For example, animal-derived 2D cell cultures cannot simulate the tissue microenvironment in vivo [10]. Additionally, they lack the complex structures, multicellular organization, and mechanical and electrical properties that are essential for supporting cell growth in vivo [11,12]. While animal models can simulate the physiological environment in vivo, there are significant differences in gene expression patterns, metabolic activity, and inflammatory cells between animal and human hearts [13]. Furthermore, cardiac drug discovery is hindered by the reliance on non-human animal and cellular models that lack sufficient throughput and physiological fidelity [14], as well as the high cost of time and money spent on preclinical trials. As our understanding of disease deepens, it is increasingly necessary to use in vitro models closer to the human body as a research platform. 3D cultured spheroids have

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* Corresponding author. Beijing Key Laboratory of Preclinical Research and Evaluation for Cardiovascular Implant Materials, Animal Experimental Centre, National Centre for Cardiovascular Disease, Department of Cardiac Surgery, Fuwai Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100037, China.
E-mail address: fwsongjiangping@126.com (J. Song).

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Abbreviations

ACM	Arrhythmogenic cardiomyopathy
AMI	acute myocardial infarction
ASC	adult stem cells
CM	cardiomyocytes
CAVD	calcifying aortic valve disease
EHT	ergonomic heart tissue
ESC	embryonic stem cells
hCO	human heart organoids
hiPSC	human induced pluripotent stem cells
SQTS	Short QT syndrome

gradually emerged [10,15,16], and more recently 3D organoids have been developed.

Organoids are three-dimensional in vitro cell constructs that summarize organ characteristics and structure to a significant extent and can mimic the in vivo structure and functional specificity of organs [17]. Organoids have many advantages over existing 2D and animal models: Compared with 2D models, they can simulate the microenvironment in the human body, and organoids can truly reflect the interaction between cells and the reaction of tissues and organs in the human body. Compared with animal models, organoid cells are derived from humans and can truly reflect human metabolic changes, and the construction is less time-consuming and requires less manpower and material resources. In addition, personalized in vitro models of organoids can be constructed from patient-derived cells [18]. Organoid studies conditions inaccessible to humans, such as embryonic and fetal development or early disease progression in adults [19]. Organoids are also used for drug screening and drug toxicity verification [14,20]. The research on organoids commenced decades ago, and numerous organoid models have been developed, including those of the liver [21], kidney [22], brain [23,24], intestine [25], stomach [26], retina [27], cochlea [28]. With the development of organoids, new drugs can be approved by the US Food and Drug Administration (FDA) without being tested on animals by legislation in 2022. Many companies have commenced the expansion of the organoid market, with organoids exhibiting considerable potential for future development.

Cardiac organoids are in vitro three-dimensional (3D) structures composed of a variety of heart cells (i.e., cardiomyocytes, endothelial cells, fibroblasts, etc.) [29]. Cells are obtained from different sources, encapsulated in a naturally derived or synthetic extracellular matrix scaffold, and given exogenous biochemical signals, such as essential growth factors [30]. Cardiac organoids have been used for disease modeling and drug cardiotoxicity screening [31], such as congenital heart disease modeling and cardiac embryonic development research [32]. Engineered heart tissue (EHT) for disease phenotypic simulation, such as studying heart disease caused by genetic mutations, including hypertrophic cardiomyopathy (HCM) caused by type B rapidly accelerating fibrosarcoma (BRAF) mutations [33], drug testing, cardiac pathogenesis research and therapeutic applications [13]. In the past, many articles have been published on organoid technology. However, these have largely focused on a certain aspect of organoid construction, and have yet to systematically summarize the construction and application of organoid platforms, particularly in cardiovascular disease research. This review aims to provide a summary of the most significant articles published over the past decade on cardiovascular organoids. It will present an in-depth analysis of the cell sources used to create organoids, the main construction schemes, the evaluation system of organoids, and their modeling and application in cardiovascular diseases. This paper presents a summary of the development of the technology, an overview of the current limitations, and a discussion of potential future developments.

2. Cardiovascular organoid construction scheme

2.1. Cardiovascular organoid cell sources

The adult heart is mainly composed of cardiomyocytes (CMs), fibroblasts, endothelial cells (ECs), and others, and the source of these cells is of particular importance for the construction of heart organoids to mimic the composition of the normal heart as much as possible. Currently, organoids can be generated from induced pluripotent stem cells (iPSCs), embryonic stem cells (ESCs), and patient-derived adult stem cells (ASCs) from normal and cancerous tissues [34,35]. Human-induced pluripotent stem cells (hiPSCs) are typically isolated from somatic cells such as fibroblasts and reprogrammed into an embryonic-like state by viral addition of pluripotent inducing transcription factors Oct3/4, Sox2, Klf4, and *c*-Myc [4]. HiPSC is reprogrammed from adult cells by instantaneously forcing the expression of exogenous transcription factor combinations. This expression can be achieved through the use of a variety of delivery systems, including non-integrated vectors, post-integration deletions, DNA-free transduction, and chemical induction [36–42]. IPSCs are readily available and are now the source of cells for most studies to build organoids because they are easy to get and commercialized. However, organoids differentiated from iPSCs tend to be immature and may require further maturation before they can be used to model adult diseases. ESCs are briefly present in early human or mouse embryos. They can be obtained directly from early embryos, but the acquisition window is short, and the operation requirements are high. So, more research is needed on constructing organoids with ESC. These ESCs have two main characteristics: infinite multiplication and pluripotency. They can proliferate indefinitely and may differentiate into any tissue in the body. ASCs are a rare pluripotent cell population with the ability to regenerate and self-renew, with high proliferation potential, and the ability to differentiate into multiple cell types, depending on the source tissue [43]. They play a role in tissue homeostasis and in replacing damaged and dead cells. ASC is produced directly from postnatal or adult tissue and derives organoids by progressively differentiating and activating various signaling pathways mediated by cytokines [35]. Given that ASC is present in the human body for a long time, the acquisition window is longer. However, the invasive nature of acquisition can be a disadvantage. Additionally, ASCs tend to be pluripotent and can only differentiate into a few types of cells, in contrast to iPSCs and ESCs, which can differentiate into a variety of human cells. The ability to proliferate and differentiate the three kinds of stem cells is an important reason for their ability to induce organoids (see Fig. 1).

2.2. Construction schemes for cardiovascular organoid

2.2.1. Tissue engineering construction

The field of tissue engineering emerged approximately four decades ago as a result of the convergence of engineering and life sciences, with the overall goal of providing biological alternatives that can replace or regenerate natural tissues lost to injury or disease [44,45]. Tissue engineering methods include the application of tissue engineering techniques and biomaterials to create 3D models of tissues and organs. These methods consist of three main components: (1) Seed cells, such as iPSC, ESC, or ASC; (2) Scaffolds to support cells: For example, the structure and composition of cardiac ECM can affect the behavior of cardiac fibroblasts, and bionic ECM substrates can enhance the differentiation and expansion of cardiac fibroblasts [46]; (3) Factors that regulate cell activity [13]. The construction of human cardiac tissue engineering mainly includes three key aspects: the establishment of the cardiac cell population, the maturation of cardiac cells and engineered tissues, and the regulation of tissue structure and function [47]. The production steps of the tissue-engineered blood vessel may include harvesting autologous cells, cell expansion, inoculating cells onto scaffolds, tissue growth, and final product testing [48]. There are now studies that have

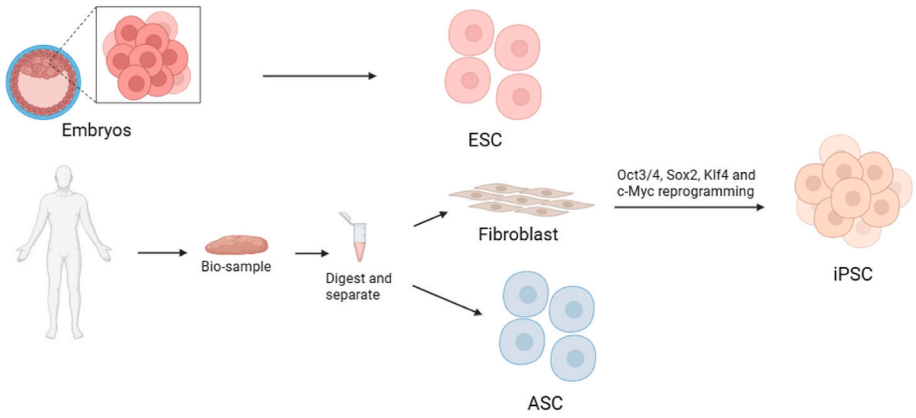

Fig. 1. Cardiovascular organoid cell sources.
Three main types of cells build cardiovascular organoids: embryonic stem cells derived from early-stage embryos, induced pluripotent stem cells reprogrammed from somatic cells such as fibroblasts, and adult stem cells isolated from adults or patients.

employed three different approaches to heart tissue engineering with moderate preclinical success: Hydrogels or fibrin gel mixtures are assembled with cells; A multilayer sheet of cells that carries its preformed matrix when implanted in cells; Cells with a broad 3D matrix structure are inoculated with biodegradable scaffolds [49]. Additionally, there are acellular methods for constructing organoids, which are designed to produce scaffolds with the ultrastructure and composition of the natural extracellular matrix (ECM) while removing all cells and genetic material present in natural tissues [50] for disease therapy. For instance, smooth muscle cells are inoculated onto a biocompatible scaffold within a disposable bioreactor, and during the culture, the cells are grown to bioengineer blood vessels. These vessels are then decellularized to produce human cell-free blood vessels [51].

Cardiomyocytes are heterogeneous groups of cells with multiple distinct ventricular, atrial, and SA node myocyte subsets. The heart is not solely composed of cardiomyocytes. It also contains pericytes, endothelial cells, and smooth muscle cells that comprise the vasculature [52]. To develop complex, transplantable, and functional cardiac tissue, it is necessary to identify suitable, scalable cell sources [53], and further integrate the differentiated types of cells to function as a unified entity. Organoid differentiation requires its unique niche signals [54]. Consequently, to induce genealogy-specific development of organoids, organoid media are often supplemented with several ligands or compounds that can activate key signaling pathways, such as transforming growth factor β (TGF-β), bone morphogenetic proteins (BMP), and the airless MMTV integration site family (Wnt), fibroblast growth factor (FGF) and Sonic Hedgehog (SHH) [55]. Different types of heart tissue can be generated through different cell combinations and proportion regulation. Previous studies have shown that EHT with the characteristics of megaloblast and atrial cell composition can be generated using collagen-based hydrogel methods [56]. The engineering of heteropolar heart tissue containing different atrial and ventricular ends has also been reported [57]. So, it is very important to establish the target heart cell population.

Because the overall goal of tissue engineering is to replace natural tissue [45], tissue-engineered myocardium needs to function like mature cardiomyocytes [49], with electrophysiological and metabolic maturity [58]. For optimal performance, engineered heart tissue is designed to be as close as possible to the natural heart muscle, thus creating an electrical and mechanical continuum [59]. Engineered heart tissue is characterized by a gene expression profile, elongated and aligned morphology, organized myotome, axial electrical connections via gap junctions, and mature action potentials and calcium transients [59]. Therefore, various cytokines [60], physical and chemical methods are required to promote the maturation of engineered tissue. 3D culture and heart-specific matrix clusters themselves can promote cardiomyocytes (CM) [61]. ERRγ treatment has been shown to stimulate the maturation of iPSC-CMs [62]. Mechanical stress can induce rhythmic CM contraction, which promotes physiological hypertrophy, cell elongation, tissue arrangement, and force generation [50]. Studies have demonstrated that the use of cyclic stretching contributes to the maturation of hESC-CMs in 3D scaffolds [49], and more extended incubation periods [63], and chronic electrical stimulation regimens can promote cardiac tissue maturation [57]. Furthermore, it has been demonstrated that blood vessel cells in human cardiac organoids (hCOs) can promote their maturation, contractility, and usefulness in disease modeling [64]. In addition, different molecular and functional approaches, such as RNA sequencing, SILAC-based proteomics, optical AP and calcium imaging, and EHM analysis, are combined to evaluate the characterization of engineered tissues [65]. It is also necessary to assess the maturity of tissue-engineered hearts from the perspectives of force-frequency relationship, morphology, electrophysiology, calcium activity, contractility, metabolic cell cycle, etc. [58] and evaluate for contractility through the open source software based on particle image velocimetry (PIV--MyoMonitor) [66], to ensure the maturity of organoids (Fig. 2).

The layered organization of cell structure is an integral part of heart function. Cardiomyocytes in the atria and ventricles have significantly different properties and support the specific functional needs of the chamber [47]. The dynamic control of tissue behavior can be achieved using microenvironment and genetic manipulation. Furthermore, the differentiation and function of stem cells can be controlled by biomaterials and modifications of biomaterials [47], thus realizing the control of organizational structure and function. Complex heart tissue structures can be manufactured by modular assembly, creating an overall structure by generating and assembling multiple simpler units [67]. In addition, CRISPR technology can also induce stem cells to differentiate into cells with specific functions [68], thus ensuring the specific and overall functional regulation of engineered tissues. The continuous development of increasingly complex technologies allows for fine regulation of the structure and function of tissue-engineered organoids (see Fig. 3).

2.2.2. 3D printing

3D bioprinting is a layer-by-layer manufacturing technique that allows precise control of tissue structure and cell distribution to replicate the complex geometry of the heart [13]. 3D printing strategies and methods mainly include stereophotolithography (SLA), digital light processing (DLP), fused deposition modeling (FDM), inkjet 3D printing, and selective laser sintering (SLS) [69]. Organ printing is defined as computer-assisted layer-by-layer additive robotic biomanufacturing of functional human 3D tissue and organ constructs using self-assembled tissues as building blocks [70].

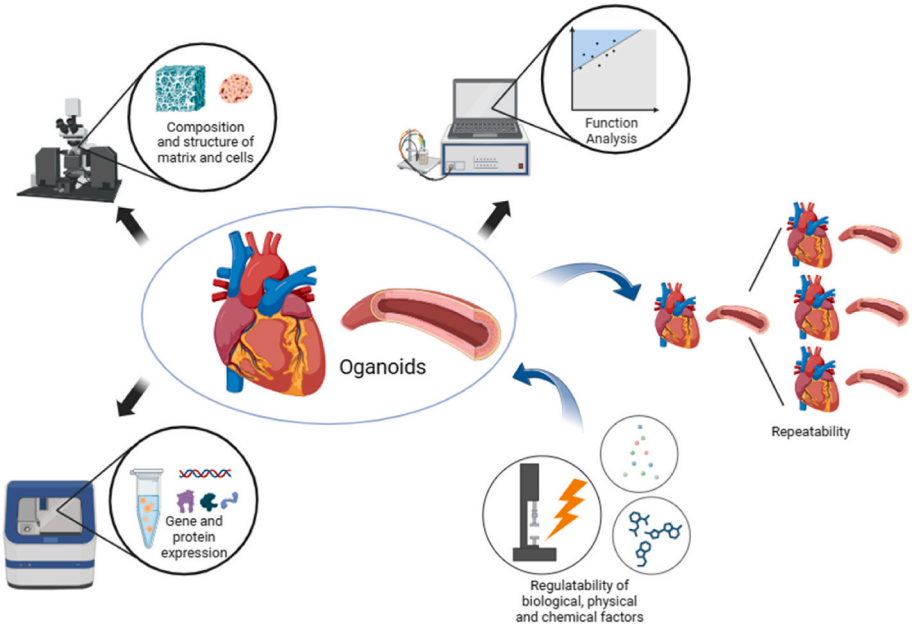

Fig. 2. Organoid evaluation.
Cardiovascular organoids were evaluated from five aspects: the composition and structure of the matrix and cells in the organoids; Organoid function; Gene and protein expression; The controllability of external biological, physical and chemical factors; And the repeatability of cardiovascular organoid construction.

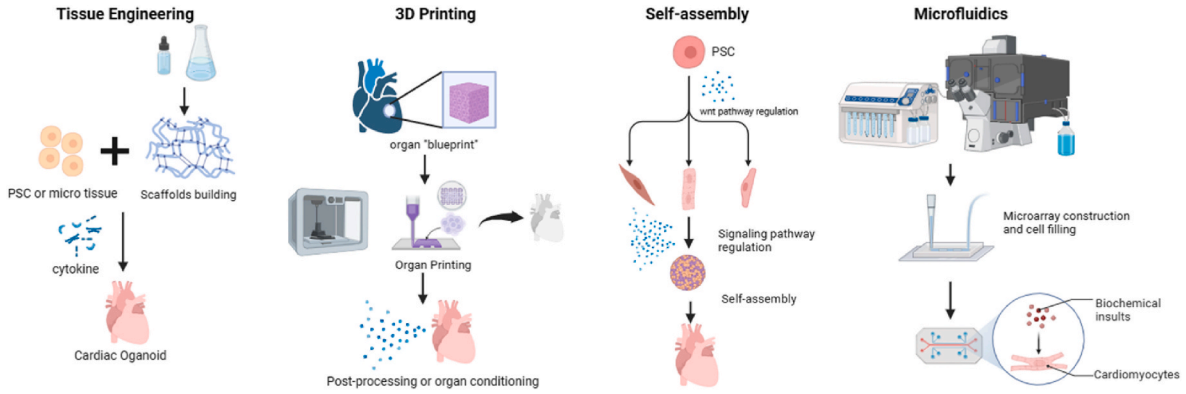

Fig. 3. Cardiovascular organoid construction program.
There are four primary construction schemes for cardiovascular organoids: tissue engineering construction that combines cells with scaffolds, 3D printing construction, self-assembly construction of stem cells induced by various chemical biological factors, and microfluidic organ chips construction.

The core of bioprinting is scaffold printing and cell printing, which consists of three consecutive steps: the design or development of the organ "blueprint"; The processing or actual organ printing; And the post-processing or organ regulation and accelerated organ maturation [71,72]. To support cell adherence and retain the structure of the artificial tissue, a 3D porous matrix was used as a scaffold into which the cells were inoculated. Ideally, scaffolds should possess the characteristics of a natural extracellular matrix, including morphological, mechanical, and biochemical properties, to provide a favorable supportive environment for cell attachment, proliferation, and tissue function formation [69]. The inherent ability of tightly placed tissue fragments or cell aggregates to fuse together is the biological basis for developing organ printing technology, assuming that aggregates of cells can fuse together to form complete tissues when placed closely in a 3D matrix [72]. Organ printing was initially used to print simple, mature blood vessels by depositing a bio-ink solution layer by layer to create vascular tissue [73], mimicking the natural counterpart, which consists of three main layers, including the outer layer, outer membrane, and inner layer [74].

With the continuous intersection of biomedicine and materials engineering, 3D bio-printed cardiovascular organoids are constantly being explored, and many 3D printing strategies have been developed. Lee et al. proposed a free-form reversible embedding using suspended hydrogel (FRESH) to successfully print simulated ventricular and trilobal valves [75]. Recently, a novel technology, embedded extrusion volumetric printing, blended extrusion bioprinting, and layer-free ultra-fast volumetric bioprinting. This technology allows for spatially realized patterns of multiple cell types [76]. Gao et al. combined 3D printing and photochemical principles, combining MPE with modulate grating scanning to induce cross-linking in a photosensitive gelatin polymer solution to generate a high-resolution ECM scaffold from the template with an architecture based on natural cardiac ECM [77]. This demonstrated the advantages of 3D printing, which can accurately confirm the shape and structure of the constructed organoids. It can also be personalized printed: cells are taken from patients, reprogrammed into pluripotent stem cells, and differentiated into cardiomyocytes and endothelial cells, and the extracellular matrix is processed into personalized hydrogels. A vascularized and perfusable heart patch was constructed by combining two cell types with a hydrogel respectively to

form a bio-ink for parenchymal heart tissue and blood vessels [78]. In addition, changes and improvements in printing materials and inks can also optimize the printing of organoids: Lee et al. found that chemically cross-linked gelatin hydrogels can regulate intercellular connectivity, enhance mechanical stability, and combine with hiPSC-CM to build good cardiac tissue organoids [79]. Microgel-based biphasic (MB) bio-inks due to their shear-thinning and self-healing behavior, by incorporating MB bioinks, the Reversible ink Template (SPIRIT) strategy enables efficient printing of ventricular models with perfusable vascular networks [80]. Kolesky et al. printed thick-walled vascular organoids from a variety of inks composed of human mesenchymal stem cells (hMSC) and human neonatal dermal fibroblasts (hNDF) along with an embedded vascular system [81]. With the continuous progress of research, more advanced 3D bioprinting technology will be used for 3D printing of heart and vascular organoids to generate simulated organoids closer to the human heart (see Fig. 3).

2.2.3. Self-assembling

Self-assembling heart organoids are usually formed by inducing aggregates of cells to differentiate and organize into heart-like structures, often requiring stem cells with high differentiation potential and the addition of specific developmental signals [13,19]. At present, there have been successful vascular organoid self-assembly schemes [82]. Kirkegaard et al. suggest that during vascular organoid self-assembly, the initial self-assembly of the closed structure is ensured by the correspondence of topic-bottom polarity (AB) to cell density, which is a crucial driver of lumen formation [83]. Cardiac organoid self-assembly requires the generation of cardiomyocytes (CMs), cardiac fibroblasts (CFs), and cardiac endothelial cells (ECs), the three main cell types in the heart, from hiPSC, and the combination of them into three-dimensional cardiac microtissues (MTs) [84].

The regulation of biological signals is essential for the differentiation and composition of cardiac cells. Hofbauer et al. established self-organizing cardioids derived from human pluripotent stem cells and found that these heart-shaped cavity morphogenesis is controlled by the mesodermal Wnt-BMP signaling axis [85]. By adjusting the Wnt pathway of the PSC, it is possible to generate cardiac organoids by self-assembly without the need for external scaffolds [86]. The cardiac organoids treated by Liang et al. with the Wnt activator CHIR99021 showed more chamber formation and more mature beating curves [87]. Similarly, the suspension embryo was sequentially exposed to the typical Wnt pathway activator CHIR99021 (inhibited by specific GSK3) and the Wnt pathway inhibitor WNT-C59 (inhibited by PORCN) at a particular time. Three successive Wnt regulatory steps to iPSC (activation/inhibition/activation) also build cardiac organoids [88]. In addition to the regulation of crucial Wnt pathways, Lee et al. demonstrated that in vitro cardiac organoids derived from mouse embryonic stem cells self-assemble into atrial and ventricular-like structures and exhibit physiological functions in the presence of lamin-internal actin (LN/ET) complexes and fibroblast growth factor 4 (FGF4) [89]. In addition to the regulation of signaling pathways, Moon et al. also found that hydrogels promote the self-assembly of vascular organoids [90].

In addition to the research into organoid construction methods, the evaluation and detection of organoids are also critical. The relative simplicity of self-assembly makes it uniquely adaptable and scalable for production engineering tissues, but inherently lacks precise control over the final shape of the network [86]. Kaushik et al. found that spontaneous fluorescence multiphoton microscopy (aMPM) can perform marker-free monitoring of vascular network morphology to simplify optimization of growth conditions and provide quality control of engineered tissues [86]. However, if cardiovascular organoids with fixed morphology and precise function are to be produced, alternative organoid construction methods or further optimization of self-assembly schemes may be required (see Fig. 3).

After the completion of cardiovascular organoid construction, the evaluation of these structures is crucial for subsequent experiments. According to the existing research, we found that there are five main aspects of assessment: The composition and structure of the matrix and cells in organoids; Organoid function; Gene and protein expression; The controllability of external biological, physical, and chemical factors; And the repeatability of cardiovascular organoid construction (Fig. 2).

2.2.4. Microfluidic organ chips

Microfluidics is a technology that allows for the manipulation of fluids through a high degree of control over the flow of fluids, with applications ranging from fluid dynamics and synthetic and analytical chemistry to biology and medicine. Microfluidic devices are tiny devices that can manipulate fluids with micron precision and are commonly used in drug development, stem cell biology, cancer biology, cell culture, and cell behavior exploration, including angiogenesis, cell migration, and cell-to-cell interactions [91]. It is also used to evaluate drug toxicity, develop drug delivery systems, regenerative medicine, and single-cell analysis [92]. Microfluidic organoids have also been used to simulate prenatal environments and explore organ development [93] and acute myocarditis induced by severe respiratory infection [94]. Microfluidic culture systems are typically made through “soft lithography,” a method of replicating patterns etched in silicon chips in a more biocompatible and flexible material [95]. The Organ-on-a-chip (OOC) platform utilizes microfluidic and three-dimensional cell culture to design miniature human tissues/organs to simulate the physiological functions of tissues and organs, with the goal not of building a complete living organ but of synthesizing the minor functional units that encapsulate functions at the tissue and organ level [96]. It can simulate human physiology and disease. The technology stems from converging advances in tissue engineering, semiconductor manufacturing, and human cell procurement [97]. It has been achieved through the fusion of advanced knowledge in cell co-culture, stem cells, genome editing, sensors, 3D printing, and microfluidics. This enables the application of biological and physical forces in a context that simulates conditions in the body [92]. The technology incorporates physiologically relevant fluid shear stress, cyclic strain, and mechanical compression levels, enabling the analysis of organ-specific responses, including the recruitment of circulating immune cells in response to drugs, toxins, or other environmental disturbances [96]. OOC can help accelerate drug development by addressing the differences in drug safety and efficacy observed between animal models, cell cultures, and clinical studies [97]. Cardiac microfluidic chips typically contain multiple microchannels and reservoirs used to culture heart cells and deliver various nutrients needed for heart cell growth. The precise delivery of reagents for controlling the development of heart cells can be achieved using microfluidic components [98].

The heart on the chip can simulate different physiological environments and conditions. The finite element method can be used to evaluate the resonance frequency of the piezoresistive cantilever of cardiac chips to measure the contractile force of cardiac cells [99]. Agarwal et al. have also increased the throughput of heart-on-chip muscle-film technology (MTF) by an order of magnitude through computer-aided design and laser-based fabrication of thin PDMS cantilever [100]. Keep pushing the limits of heart chip research. Marsano et al. developed a heart-on-a-chip platform that Outlines the physio-mechanical environment experienced by cells in the natural heart muscle [101]. Many teams continue to explore new technologies for the construction and application of cardiovascular organoids: Sokolowska et al. proposed a digital controlled differentiator integrated with a cardiac system on a chip using a newly developed digital controlled differentiator for stem cell differentiation and full-functioning cardiomyocyte generation [102]. Myocardial interstitial fibrosis represents an essential component of cardiac remodeling, which alters myocardial structure and function, leading to heart failure and death. Mastikhina et al. designed a microfluidic chip to explore the fibrosis process [103]. The diverse platform design and critical design capabilities of organ-on-a-chip allow for a wide range of data readings that can be used for computational modeling as part of the

drug discovery process. The wide diversity of tissue platforms highlights key common features, including three dimensions of tissue culture, encompassing multiple cell types, and biomechanical modeling that reconstructs the environment in vivo [104]. An in vitro vascularized human heart microtissue (MT) model was constructed based on human induced pluripotent stem cells (hiPSC) in microfluidic organ chips by co-culturing hiPSC-derived, pre-vascular heart MT with vascular cells in fibrin hydrogels [105]. 3D cardiac MTs can be integrated into microfluidic chips via an external vascular network formed by hiPSC-EC and HBVPs [105].

Furthermore, vascular microfluidic chips can simulate the physiological environment and different stimuli, so a lot of research has been done on the influence of vascular mechanical force, the role of inter-vascular paracrine [106], and the role of inter-vascular paracrine [107]. For example, microfluidic flow stretching chips that integrate fluid shear stress (FSS) and cyclic stretching (CS) can simultaneously or independently deliver FSS and CS to blood vessel cells to simulate the hemodynamic microenvironment of blood vessels in the body [108]. In addition, numerous vessels on a chip have been developed to simulate various cardiovascular diseases, such as atherosclerosis, arterial thrombosis, and plaque formation [91]. Costa et al. developed a method for fabricating microfluidic chips containing small blood vessel structures that closely resemble those found in healthy and narrow blood vessels [109]. The heart and vascular organoids designed by microfluidic can simulate various physiological and pathological states. Additionally, they can be utilized for physical and chemical factors and read relevant data through fine regulation, which can be widely used in cardiovascular physiology, disease research, and drug screening (see Fig. 3).

2.2.5. Summary of four construction schemes

The advantage of tissue engineering construction is that materials can be used to bind to cells, and organoids can be constructed in simple shapes. The construction difficulty of a tissue engineering construction scheme is moderate, and organoids of relatively definite shape can be built, although the accuracy is not high. It is mainly used in the construction of organoids for the treatment of cardiovascular diseases and disease models, such as the construction of heart patches and the replacement of transplanted blood vessels. However, the impact of innovative materials used in organoid construction on organoids should be considered as one of the critical factors in organoid construction. 3D printing is primarily used to construct organoids with specific myocardium or vascular tissue forms. The construction scheme is complex, and the adjustment of parameters mainly relies on repeated trials, which is time-consuming. However, organoid structure and morphology can be precisely regulated, and highly complex organs can be printed [110]. The advantage of self-assembly construction scheme to construct organoids is that it is relatively straightforward to operate. However, the shape and regulation of organoids are rough, and the size of the formed organoids is often relatively small. The microfluidic chip can easily and accurately regulate cardiovascular organoids' biological, physical and chemical parameters. However, organ chips are mainly used for drug development and adjustment of physiological and pathological factors, and there is no organ entity. The appropriate organoid construction scheme can be selected according to the platform, construction purpose, construction precision, and regulation requirements.

2.3. Cardiovascular organoid disease modeling

2.3.1. Cardiomyopathy model

Hypertrophic cardiomyopathy (HCM) is a leading cause of heart failure and sudden cardiac death and is generally undetected in the general population [18,111]. Studies in cardiac organoids have shown that mechanical stress can control the hypertrophy and structure of engineered human myocardium [112]. This approach contributes to studying new treatments simply and rapidly [113]. Animal TAC models make distinguishing direct load-induced changes from systemic and humoral mechanisms difficult. EHT reflects human myocardial tissue characteristics and simulates preload-induced cardiac hypertrophy, a common cause of heart hypertrophy. In addition to mechanical stress, biochemical factors are also important causes of hypertrophic cardiomyopathy: Ma et al. used two-photon polymerization (TPP) to prepare filamentous substrates combined with iPSC to construct cardiomyopathy in vitro models. Their findings indicated that myosin binding protein C (MYBPC3) deficiency and mechanical overload could lead to contraction defects in cardiomyopathy-engineered heart microtissues [114], which may contribute to HCM. A cardiac platform on a chip developed by Marsano et al. provides a standard functional three-dimensional model of the heart that may predict signs of phenotypic hypertrophy changes through mechanical and biochemical co-stimulation, such as isoproterenol [101]. It should be noted that HCM is also prevalent in patients with heart-facial-skin syndrome (CFCS), a genetic disorder characterized by abnormal signaling in the RAS/MAPK signaling cascade [18]. Cashman et al. developed the first 3D ergonomic heart tissue (hECT) model of HCM: This was obtained by directed differentiation of induced pluripotent stem cells from CFCS patients due to activated BRAF mutations, which for the first time outlined key features of the HCM phenotype [18]: BRAF-hECT showed a hypertrophic phenotype on the sixth day of culture, with elevated tissue size, and atrial natriuretic peptide gene expression. Organoids reduce the biological complexity of the system to a manageable number of controllable factors and maintain high biological fidelity through direct measurement of electromechanical muscle function compared to cellular models [18]. In addition, MYBPC3, a cardiac subtype, is a coarse filament helper protein in the A band of striated muscle, and MYBPC3 mutation is associated with HCM [115]. Gene editing can also facilitate the construction of HCM organoids. For instance, iPSC-CM exhibits an HCM phenotype following MYBPC3 knockout via CRISPR/Cas9 [116], which can be used to construct HCM cardiac organoids.

Another area of organoid modeling is dilated cardiomyopathy (DCM). Human mutations that truncate large amounts of myotome protein titers [TTN truncation variants (TTNtvs)] are the most common genetic cause of dilated cardiomyopathy. Studies have been conducted on heart microtissues constructed by hiPSC through gene editing and tissue engineering techniques to evaluate the pathogenicity of titin gene variants [111]. The possible mechanisms underlying TTNtvs-induced myotome insufficiency, impaired response to mechanical and β-adrenergic stress, and decreased activation of growth factors and cell signaling leading to DCM were elucidated. Streckfus-Bomeke et al. generated iPSCs from a patient with dilated cardiomyopathy (DCM) caused by the malposition mutation S635A in RNA-binding motivation protein 20 (RBM20), and engineered heart muscles (EHMs) from RBM20-IPSC-CMS showed that not only the main force production of RBM20-EHMs is impaired, but the passive stress of the tissue is also reduced, which indicates that RBM20-EHMs has higher viscoelasticity [117]. Compared with the cell model, the DCM organoid model has higher maturity and more faithfully reflects the response of cardiomyocytes to drugs. Their study highlights the utility of the bioengineered iPSC-CM platform in drug testing.

Arrhythmogenic cardiomyopathy (ACM) is an inherited heart disease characterized by pathological fat infiltration and loss of cardiomyocytes, primarily in the right ventricle [118]. Kim et al. generated iPSC lines from fibroblasts from two patients with ARVD/C and PKP2 mutations and established an effective in vitro model of arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) [119]. Abnormal peroxisome proliferator activated receptor γ (PPAR-γ) activation was found to be the basis of ARVD/C pathogenesis [119]. The iPSC line, which can be used to construct organoids, is derived from patient cells and can realistically reflect the state of the myocardium in the patient's body. Goldfracht et al. developed a model of arrhythmogenic cardiomyopathy based on atrial EHT to study reentrant arrhythmias, focusing on atrial arrhythmias, such as AF, and confirming their effectiveness by applying

relevant pharmacological interventions [120].

We found that cardiac organoids are widely used in HCM, DCM, and ACM, providing a more accurate in vitro model for various typical cardiomyopathies and facilitating the study of disease mechanisms and progression. It can also offer personalized in vitro modeling for patients to explore treatment options. In comparison to existing cell models and animal models, cardiac organoid models demonstrate unparalleled advantages: It is easy to control a single variable and eliminate the influence of multiple systems in animal models. It is more similar to the human heart and better reflects the human heart's response to external variables. It can directly monitor the heart organoids with less interference and higher data fidelity. Finally, organoids generated from patient-derived cells are specific and can be personalized to reflect the characteristics of the patient's heart, thus facilitating the identification of personalized treatment options.

2.3.2. Myocardial ischemia model

Ischemic heart disease (IHD) is the most prevalent cardiovascular disease and the leading cause of mortality worldwide. In IHD, blood flow to the heart muscle is reduced or blocked, leading to oxygen and nutrient deprivation, as well as the accumulation of metabolic waste in the tissues, leading to damage and death of heart muscle cells [121]. IHD mainly consists of atherosclerotic coronary artery obstruction or narrowing resulting in myocardial ischemia, such as myocardial infarction (MI).

During acute myocardial infarction (AMI), ischemia/reperfusion (I/R) damage leads to the death of cardiomyocytes and loss of tissue function, making AMI one of the leading causes of death worldwide [122]. Sebastiao et al. used CM (hiPSC-CMs) self-assembly derived from human induced pluripotent stem cells to establish a novel human in vitro model of myocardial I/R injury that Outlines important features of AMI, including loss of CM activity and destruction of cell ultrastructure. Increased angiogenic potential and key pro-angiogenic and pro-inflammatory cytokine secretion [122]. Self-assembled myocardial infarction cardiac organoids containing oxygen diffusion gradients and stimulated by the neurotransmitter norepinephrine have been studied [123]. It modeled the structure of the human infarcted heart (by simulating infarcts, boundaries, and remote areas), and the general features of myocardial infarction were generalized at the transcriptomic, structural, and functional levels (especially pathological metabolic metastasis, fibrosis, and calcium treatment). It was found that organoids can simulate hypoxic-enhanced adriamycin cardiotoxicity [124]. Although mouse models have been widely used, there are differences between mouse and human cell physiology at the tissue and cellular level, including variations in ion channels [122]. Consequently, organoids reflect the inflammatory response and drug effects of human heart muscle better. The in vitro myocardial infarction model constructed by cardiac organoids can simulate the disease state well and can be easily used in I/R modeling, which provides a good platform for intuitive research on the progression of myocardial infarction and the treatment of I/R. It also eliminates the effects of complex systems in the body.

2.3.3. Cardiovascular injury model

Cardiovascular injury due to various reasons is widespread in clinical practice. One study has used human embryonic stem cells to construct cardiac organoids through tissue engineering, developed and characterized in vitro models of human hearts with acute freezing injury, and found that regenerative ability is an intrinsic characteristic of immature human heart tissues [125]. J. Yang et al. studied vascularized hCO and found that vascularized CO cardiomyocytes contracted asynchronously, with fibrosis formation and cTnT releasing during freezing injury [126]. While respiratory symptoms are typical of COVID-19, patients experience heart damage and dysfunction and an increased risk of death [127]. Furthermore, SARS-CoV-2 infection has been found to cause multi-spectrum heart damage in 3D cardiac organoids, demonstrating the potential of organoids in studying the effects of viral infections on the heart [128]. The combination of human heart organoids with phosphorylated proteomics and mononuclear RNA sequencing determined that the inflammatory "cytokine storm" induced diastolic dysfunction caused by COVID-19 infection could be mitigated by bromodomain and extra terminal (BET) family inhibition [127,129]. SARS-CoV-2 infection can also cause vascular damage, and both human and rhesus monkeys infected with SARS-CoV-2 showed endothelial destruction and thrombosis [130]. Kawakami et al. developed a progenitor cell-derived human vascular organoid model with SARS-CoV-2 infection capacity and revealed the role of complement factor D (CFD) in mediating microvascular immune thrombosis [131]. In addition, the mechanisms underlying drug-induced heart damage are still unknown. For example, the specific mechanism of heart damage after treatment with many tumor chemotherapy drugs (anthracyclines, monoclonal antibodies, etc.) is unknown [132]. Therefore, simulating drug-induced cardiac injury through cardiac organoids will be helpful for the study of related mechanisms and the exploration of treatment [133]. Cardiac organoids constructed from human-derived cells can truly reflect all kinds of stress, immune response, and signal pathway changes after heart injury in the human body, which is convenient for exploring related treatment schemes.

2.3.4. Congenital cardiac disease model

Congenital heart defects are the most common human congenital disabilities, but our understanding of the origin of these disorders limits our knowledge of the origins of these disorders. However, research has now found a way to generate developmentally relevant human heart organoids by using the self-assembly of human pluripotent stem cells, which can be used to simulate the characteristics of congenital heart disease induced by pre-gestational diabetes [88]. A team constructed a cave-specific organoid composed of major heart cell types, used this model to analyze organoid cells from genotypic lines in NKX2-5 that carry genetic variants associated with Ebstein abnormality, and successfully generalized the auricular ventricular defects of the disease: Heterotopic expression of smooth muscle gene MYH11 and defective myotome structure were found in the mutant. Additionally, the number of atrial markers (MYH6, ID2) in ventricular organoids was high, while the number of ventricular markers (MYH7, HEY2) was less than expected [134], to simulate standard and diseased heart development. Compared with animal models such as zebrafish, organoids can more intuitively observe the differences in heart development, and it is easier to monitor and intervene in genes and cell products. Cardiac organoids visually display the difficult-to-observe embryonic heart development, which is convenient for understanding the key processes in heart development and the important nodes leading to the progression of the disease to conduct an efficient intervention, personalized treatment, and reduce the incidence of congenital heart disease.

2.3.5. Arrhythmia model

Arrhythmia is a common cardiovascular disease. It is essential to study its mechanism and treatment. To this end, there have been studies using iPSC to establish patient-specific hiPSC atrial cell and tissue models of Short QT syndrome (SQTS), and it has been found that the use of hiPSC derived atrial cell and tissue models can be extended to study other inherited or acquired atrial arrhythmias [135]. A team has also combined human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) with chitosan-enhanced extracellular matrix (ECM) hydrogels derived from decellular pig hearts to construct ECM-derived engineered heart tissue (ECM-EHT) [136]. This can be used to study the electrophysiological and calcium-processing properties of ECM-EHT to detect drug-induced contraction rates such as isoproterenol, cellular arrhythmia, and tissue conduction properties [136]. A similar assay in ECM-EHT derived from patient-specific hiPSC-CM Outlines the abnormal phenotypes of long QT syndrome and catecholamine-sensitive pleomorphic ventricular tachycardia, with programmed electrical stimulation and drug-induced proarrhythmic disorders leading to the

development of reentrant arrhythmias in ECM-EHT [136]. Compared with cell culture, the three-dimensional environment, multicellular interactions, extracellular matrix, and applied mechanical and electrical stimulation in cardiac tissue engineering make hiPSC-CMs more mature. As a 3D model, it can also better monitor the electrical activity of the myocardium, which is more conducive to the mechanism of arrhythmia research and drug development. Human cell-derived heart muscle better reproduced the electrical activity of human heart muscle cells. Compared to animal models, the ion channel responses and changes in organoids help us get closer to the truth of arrhythmia. And the fundamental pathway changes make it possible to find targeted drugs.

2.3.6. Normal and valvular disease models

Valvular heart disease (VHD) is an essential cause of loss of function, quality of life, and longevity, mainly due to functional and degenerative diseases and rheumatic heart disease [137]. The primary treatment for end-stage valve disease is valve transplantation, and mechanical, biological, and non-degradable polymer valves have been developed to ensure long-term function after implantation [138]. Scaffolds for valve tissue engineering can be broadly divided into two categories: synthetic biodegradable polymers and natural substrates [139]. One team constructed a bioabsorbable polyε-caprolactone (PCL) heart valve prosthesis by electrospinning [140]. Dube et al. induced extracellular matrix protein production and assembly by cultivating dermal fibroblasts in ascorbate-containing media and constructed valvular tissue sheets [141].

In addition to valvular organoids for therapeutic purposes, there are also models for mechanism studies. The pathogenesis of calcifying aortic valve disease (CAVD) is far from fully understood. It is hypothesized that this is due to valve damage and subsequent activation of cell repair damage while also involving reactivation of developmental processes such as endothelium-to-mesenchymal transformation (EMT) [142]. A comprehensive understanding of CAVD will allow the development of suitable trim molecule/drug treatment options for this disease. A representative 3D model of the aortic valve could shed light on this knowledge by identifying and understanding cellular and ECM changes in early disease states [143] and could also further advance the treatment of valvular disease. The application of organoids in valvular modeling allows us to understand better the pathology and disease progression of valvular diseases such as CAVD, which can be further used to study common valvular diseases such as rheumatic heart valve disease in the future.

2.3.7. Normal and vascular disease models

As an integral component of the cardiovascular system, blood vessels are susceptible to numerous diseases that significantly threaten life and health. Consequently, research on vascular organoids is also continuing. Nie et al. combined a new method of fabricating hydrogel-based microfluidic chips with casting and bonding processes to establish an on-chip vascular system with vascular function under physiological and pathological conditions [144]. Blood flow within blood vessels is typically laminar and streamlined. However, when the geometry of blood vessel changes abruptly due to branching, sharp turns, or narrowing, the EC will experience low-shear perturbation flow, which can lead to endothelial dysfunction and atherosclerosis. Tovar-Lopez et al. developed a microfluidic system with integrated ridged obstructions to generate controllable perturbed flow patterns to study the effects of perturbed flow on cytoskeletal remodeling and nuclear shape and size of cultured human aortic endothelial cells [145].

Tissue engineered blood vessels (TEBVs) can be used clinically for coronary artery bypass grafting, peripheral artery disease, or as arteriovenous grafts for hemodialysis access [143,146]. Growing living tissue-engineered vascular grafts (TEVG) have been developed and shown to increase with age while maintaining full function in large animals [147]. Analysis using novel CT imaging tools revealed no dysfunctional degeneration of the vascular graft, such as calcification, stenosis, thromboembolism, and graft dilation or aneurysm formation [147]. Blood vessels composed of smooth muscle cells and extracellular matrix proteins are formed by human tissue engineering, and these smooth muscle cells are inoculated on a biodegradable scaffold using a biomimetic perfusion system to generate blood vessels by decellulating and suturing lines, providing an alternative to small-diameter vascular grafts [148]. Cell-free tissue-engineered blood vessels have been used in human trials to develop an investigatory bioengineered human acellular blood vessel (HAV), which acts as a hemodialysis catheter for patients with end-stage renal disease, and studies have found that HAV exhibits host myogenic properties, making these vessels resemble the patient's own blood vessels [149]. Shi et al. constructed a three-layer vascular graft with a natural arterial decellularized extracellular matrix (dECM) that mimics arterial components [150]. Salidroside is loaded into the inner layer of the vascular graft to inhibit thrombosis. Vitro studies have shown that dECM provides a bioactive environment for extended adhesion, proliferation, migration, and tube formation of human umbilical vein endothelial cells (HUVEC). In vivo studies have shown that adding dECM can promote endothelialization, smooth muscle regeneration, and extracellular matrix deposition [150].

Aortic disease is defined as a condition that affects any part of the aorta, including the aortic arch, thoracic aorta, and abdominal aorta. It involves focal dilation of blood vessels due to structural aortic disease (aneurysm, dissection, or rupture), atherosclerosis, or other connective tissue diseases (Marfan syndrome, Ehlers-Danlos syndrome) [151]. Due to the lack of effective drug treatment for aortic aneurysm, the main treatment is surgical treatment. In order to further study the treatment of aortic aneurysm, a research team designed an aortic smooth muscle chip to simulate human aortic physiology and pathophysiology for further drug screening [152,153].

Diabetes is a leading cause of blindness, kidney failure, heart attack, stroke, and lower limb amputation, often due to diabetic vasculopathy [154,155]. By inducing iPSC to self-assemble into a capillary network wrapped by a basement membrane and performing in vitro diabetic vascular variant modeling, DLL4 and NOTCH3 were found to be critical drivers of diabetic vascular disease in human vessels [154]. It provides a new direction for the treatment of diabetic vascular diseases and reflects the great potential of organoids in the study of vascular diseases. As an important part of the circulatory system, it is essential to study and treat the mechanism of vascular diseases. Different vascular organoids, including small vessels, vascular networks, arteries, etc., provide more possibilities for the study of mechanisms and treatment of arteriosclerosis, aneurysms, arterial dissection, and diabetic vascular disease.

2.3.8. Organoids combined with CRISPR/cas technology

In addition to being a tool for introducing genetic variation into iPSCs, CRISPR/Cas9 can also effectively correct genetic variation in iPSCs [156]. Wang et al. developed a protocol based on doxycycline-induced Cas9 transgene carried on piggyBac transposons to enable robust and efficient Cas9-directed genome editing so that parental lines can be rapidly designed to carry many individual mutations [157]. This approach allows efficient, targeted genome editing and simultaneous scar-free transgene excision editing [157]. It has contributed to the diversification of organoids. Short QT interval syndrome (SQTS) is a genetic disorder. A patient-specific hiPSC-CM was used to generalize the SQTS disease phenotype at the cellular and tissue levels. Subsequently, mutations in the KCNH9 gene in cardiac organoid cells of SQTS patients were corrected using CRISPR/Cas2 gene editing technology, which could produce normal cardiac organoids [158,159]. The results demonstrate the potential of CRISPR/Cas technology in organoid research.

We found that in the above seven categories of disease modeling, most of the use of tissue engineering, self-assembly, and microfluidic organ chips, almost no 3D printing (Table 1). For this, first, 3D printing technology is complicated and expensive. In addition, we think that the possible explanation is that we are generalizing models of various

Table 1
Cardiovascular organoid disease modeling summary.

	Disease Type	Construction Method	Research Contents	Reference
Cardiomyopathy model	Hypertrophic cardiomyopathy	Tissue engineering	Effect of mechanical stress on human cardiac hypertrophy	[112]
	Hypertrophic cardiomyopathy	Tissue engineering	Cardiac hypertrophy due to increased afterload	[113]
	Hypertrophic cardiomyopathy	Tissue engineering	MYBPC3 lacks effect on HCM	[114]
	Hypertrophic cardiomyopathy	Organs-on-chips	Combined mechanical and biochemical stimulation to predict cardiac hypertrophy	[101]
	Heart-facial-skin syndrome	Self-assembly	Key features of HCM phenotype	[18]
	Hypertrophic cardiomyopathy	Tissue engineering	Possible mechanisms of TTNtvs leading to HCM	[111]
	Dilated cardiomyopathy	Tissue engineering	The molecular mechanism of RBM20-dependent pathological cardiac remodeling leading to DCM was revealed	[117]
	Arrhythmogenic cardiomyopathy	Self-assembly	PPAR-γ activation is the basis of ARVD/C pathogenesis	[119]
	Arrhythmogenic cardiomyopathy	Tissue engineering	Atrial tachyarrhythmia	[120]
Myocardial Ischemia Model	Acute myocardial infarction	Self-assembly	Myocardial I/R injury	[122]
	Myocardial infarction	Self-assembly	Simulates the structure of a human infarcted heart	[123]
	Myocardial infarction	Self-assembly	Simulated hypoxic enhanced doxorubicin cardiotoxicity	[124]
Heart Injury Model	Acute freezing injury	Self-assembly	Regenerative capacity is an intrinsic property of immature human heart tissue	[125]
	Freezing injury	Self-assembly	Freezing injury myocardial fibrosis and cTnT release	[126]
	COVID-19 infection injury	Self-assembly	BET inhibition is a key mechanism to mitigate the effects of cytokine storms	[127]
	Drug-Induced injury	Tissue engineering	Heart organoids to simulate drug-induced heart damage	[133]
Congenital Heart Disease Model	Congenital heart disease	Self-assembly	Characteristics of congenital heart disease induced by pregestational diabetes	[88]
	Congenital heart disease	Self-assembly	Atrial ventricular defects carrying genetic variants associated with Ebstein abnormality in NKX2-5 are summarized	[134]
Arrhythmia Model	Short QT syndrome	Self-assembly	Short QT syndrome	[135]
	Long QT syndrome, ventricular tachycardia	Tissue engineering	Programmed electrical stimulation and drug-induced arrhythmia	[136]
Valve Model	Valvular sheet	Self-assembly	Valvular sheet	[141]
	Calcifying aortic valve disease	Self-assembly	Identify and understand early disease states	[143]
Vascular Model	Physiological and pathological conditions of the vascular system	Organs-on-chips	An on-chip vascular system with vascular function in physiological and pathological conditions	[144]
	Low shear disturbance flow	Organs-on-chips	To study the effect of perturbed flow on cultured human aortic endothelial cells	[145]
	Arteriovenous graft	Tissue engineering	Living autologous tissue engineering vascular grafts with the ability to grow	[147]
	Small blood vessel graft	Tissue engineering	Small diameter vascular graft	[148]
	Human cell-free blood vessels	Tissue engineering	Hemodialysis catheter for patients with end-stage renal disease	[149]
	Vascular graft	Tissue engineering	The addition of dECM can promote endothelialization, smooth muscle regeneration, and extracellular matrix deposition	[150]
	Human aorta	Organs-on-chips	Simulation of human aortic physiology and pathophysiology	[152, 153]
	Capillary network	Self-assembly	In vitro diabetic vascular change model	[154]

The table summarizes the models of cardiovascular organoids constructed in different diseases, including cardiomyopathy, myocardial ischemia, heart injury, congenital heart disease, arrhythmia cardiomyopathy, valve, and blood vessel models, as well as each model's construction methods and research content.

cardiovascular diseases, simulating multiple pathological states, including cell necrosis, matrix changes, and structural changes in the disease state. At present, the organoids that can be built by 3D printing are mainly in a normal state. Due to the comprehensive construction of morphology using materials such as gelatin in the printing process, although morphology and function are close to the heart, the intercellular artificial material matrix within it may limit the routine progression of the disease. Consequently, 3D-printed organoids are less commonly used for disease modeling, and more used for disease treatment rather than disease modeling. In order to ascertain the underlying reasons for this phenomenon, further research is required.

3. Application of cardiovascular organoids

3.1. Cardiovascular organoids are employed for drug screening

Drugs for cardiovascular disease are still limited. Drug screening of human heart organoids is more effective than 2D models because it eliminates the false positives detected in traditional 2D culture forms and predicts potential side effects on cardiac contractility and rhythm [160]. Mills et al. found drug inconsistencies between the hCO system and traditional 2D essences. They used high-throughput proteomics in hCO to reveal synergistic activation of mevalonate pathways and cell cycle networks by proliferative compounds [160]. The team used engineered human ventricular-like cardiac tissue strips and organoid chambers to test the pharmacological responses of 25 cardioactive compounds across a variety of drug classes [161]. Combined with induced pluripotent stem cell technology, this human heart tissue platform can provide patients with a specific model for personalized drug screening for optimal therapeutic application. Zhu et al. identified potential drugs such as digoxin and 2-methoxyestradiol for the treatment of thoracic aortic aneurysms by developing a high-throughput aortic smooth muscle chip model for drug screening [152]. Chen et al. also established an in vitro model of the micro physiological aorta associated

with microfluidic technology to detect the drug action of metformin in the system [153]. Studies have shown that BETi can reduce transcription of genes involved in viral response, decrease ACE2 expression, and reduce SARS-CoV-2 infection in cardiomyocytes in the heart injury caused by COVID-19. The BD-2-selective compound RXV-2157 and the U.S. FDA-designated drug apabetalone were found to alleviate myocardial damage and SARS-CoV-2 infection [127,129]. Other studies have found that IPSC-derived human capillary organoids with high levels of ACE2 expression are susceptible to SARS-CoV-2 infection, and human recombinant soluble ACE2 treatment can protect these vascular organoids from infection [162].

3.2. Cardiovascular organoids are employed for drug toxicity detection

The use of drugs can affect the function of the heart. However, due to differences in drug response between animals and humans, drugs in animal models do not reflect their actual reactions in humans [163]. Using animals as preclinical experimental models can easily lead to the situation that drug side effects cannot be presented in reality. Cardiovascular organoids can better simulate the human internal environment and more truly react to drugs, so they are used for in vitro studies of different drug reactions. Some studies that used a bio-3D printer to fabricate stent-free 3D tubular heart structures developed a method to measure contractility, evaluate drug response in the heart structure, and measure contraction and cell viability of the heart structure after medication [163]. Another study accurately characterizes cardiotoxic damage caused by the anticancer drug doxorubicin through self-assembled cardiac organoids [164]. It has recently been found that hiPSC-CMs can be used to simulate antitumor drug-induced cardiotoxicity in vitro [165], which may provide more recommendations for future use of antitumor drugs. For example, the chemotherapy drug mitoxantrone can cause arrhythmias, and studies in cardiac organoids have found that co-administration of metformin can partially reverse this effect [166]. In addition, by coating the surface of hiPSC-CMs with extracellular matrix proteins to generate 3D tissue in a short time, the effects of several compounds with different mechanisms of action (e.g., ouabain, pimobendan) were examined through cell motion imaging. Human ether-relevant gene (HERG) channel blockers with a high risk of arrhythmia were found to cause prolonged contractile-relaxation duration and arrhythmia-like waveforms [167]. One team has developed an in vitro vascularized human heart microtissue (MT) model based on a microfluidic organ microchip, by co-culture the HIPSC-derived pre--vascular MT of the heart with vascular cells in a fibrin hydrogel [105]. This platform lays the foundation for studying how the organ-specific EC barrier responds to drug or inflammatory stimuli.

3.3. Cardiovascular organoids are employed for testing the effectiveness of gene therapy

Cardiovascular organoids are also used to study gene therapy for some inherited cardiovascular diseases. Wang et al. combined patient-derived induced pluripotent stem cells (iPSCs), genome editing, modified RNA (modRNA), and organ-on-a-chip models simulating cardiac tissue to replicate the pathophysiology of BTHS cardiomyopathy in tissue constructs [168]. The study demonstrated that the BTHS cardiomyopathy phenotype is easily reversed by reintroducing wild-type (WT) TAZ or inhibiting excess reactive oxygen species (ROS) by BTHS mitochondria. In addition, Sleiman et al. modified patient-derived induced CM in patients with pleomorphic ventricular tachycardia caused by RyR2-H29D mutation through gene editing, resulting in a shortened action potential and the restoration of typical arrhythmias and contractions [169,170].

3.4. Cardiovascular organoids are employed in cardiovascular physiology and development research

Due to the particularity of the human heart, many of its physiological characteristics have not been thoroughly studied, so organoids are also widely used in the study of cardiac physiology. One study integrated heart organoids into dual photomultiplier tube (PMT) sensors that can simultaneously measure oxygen, field potential, and contraction in real time. The simultaneous electro-metabolic mechanical sensing demonstrated that mitochondrial function in human heart organoids is synchronized with their electrical activity [171].

Cardiogenesis is a complex process that requires intercellular signaling or interactions between tissues to ensure the normal progression of the developmental process. The emergence of cardiac organoids also provides a way to explore key drivers of the heart's development to maturity [158]. By reflecting the early stages of cardiac development, cardiac organoids provide a new way to study the mechanisms of heart malformations associated with early embryogenesis. Hofbauer et al. recreated the process of early heart development in cardiac organoid self-assembly using hiPSC, revealing that Wnt-BMP is involved in the formation of heart chambers [85]. One study has used tissue engineering to embed human pluripotent stem cell aggregates in the matrix gel and then conducted directional cardiac differentiation through small-molecule biphasic WNT pathway regulation to generate highly structured three-dimensional heart-forming organoids for the study of early cardiac development patterns [172]. The establishment of cardiac organoids provides a method for studying the different developmental stages of the heart [158]. One study describes a self-organizing human multilineage organoid that reproduces the co-emergence of the anterior epicardium, septal transverse mesenchyme, and liver buds [173]. These organs include an epicardium-like layer that completely surrounds the myocardium-like tissue. These cardiac organoids outline the influence of epicardium cells on promoting cardiomyocyte proliferation and structural and functional maturation [173]. Cardiac maturation after birth is also very important. In the in vitro hCO study by Mills, R.J. et al., it was found that the transformation of CM into fatty acid metabolism was the core driver of cardiac maturation. Activating beta-catenin and YAP1 can promote heart maturation [174]. In addition, the multi-chamber heart organoids reveal the ontology of signal and contraction propagation between interacting heart chambers and analyze how mutations, teratogens, and drugs cause specific compartment defects in the developing human heart [175]. For example, microplastics have been shown to affect human health, which could induce cardiac hypertrophy [176]. Polystyrene nanoplastics (*PS*-NPs) found in self-assembled cardiac organoids not only cause the cytotoxicity of hESCs in a dose- and time-dependent manner, but also hinder cardiac differentiation, manifested by low differentiation efficiency, deformities, and weak contraction at CM and CO levels [177]. The use of cardiac organoids facilitates a deeper understanding of the processes involved in heart growth and development.

3.5. Cardiovascular organoids are used for disease treatment

Several constructed cardiovascular organoids have the potential to be used in disease treatment. For example, while the utility of cardiac patches in clinical applications has not been demonstrated, engineered myocardial patches in repairing small and large animals with infarcted hearts often result in reduced scar size, increased wall thickness, and modest improvements in left ventricular function parameters [53]. In this study, a heart patch was designed to treat myocardial ischemia by using tissue engineering to create an acellular porcine small intestinal submucosal extracellular matrix (SIS-ECM) patch implanted with iPSC-induced human cardiovascular progenitor cells (hCVPC) and cardiomyocytes (hCMs) [178]. In addition, cardiac organoids designed with electrically conductive silicon nanowires (e-SiNW) can improve the electrical pacing capacity of CMs, enabling effective cardiac repair

[179]. Cosentino et al. provide a novel experimental tool called 3D eX in Vivo Muscle Engineered Tissue (X-MET) to define the contribution of mechanical stimulation in triggering functional remodeling and saving myocardial ischemia [180]. Biomechanical stimulation-induced cardiac remodeling of X-MET, suggesting a potential therapeutic product for developing new strategies for regenerative medicine in myocardial ischemia. Svystonyuk et al. used acellular biological extracellular matrix scaffolds to stimulate muscle repair pathways and restore tissue function, and studies have shown that biological scaffold therapy is associated with improved infarcted myocardial perfusion, reduced myocardial scar load, and reverse structural remodeling [181]. Kook et al. used a multi-scale scaffold to reconstruct cardiovascular tissue, dividing cell compartments into the core region of heart tissue and the peripheral area of blood vessels to construct cardiovascular tissue, and the resulting scaffolds promoted the formation of new blood vessels of ADSCs and the expression of myocardial phenotypes [182]. Multi-scale stents are expected to be used for the remodeling and transplantation of cardiovascular tissue. In addition, many complex functional hydrogels have been utilized in the repair of myocardial ischemic injury [183].

A variety of approaches have been introduced to design ventricle-like structures and the entire heart, including decellularization. A complete whole-heart stent can be obtained by injecting the natural heart through the coronary vasculature, removing the cells with chemical and biological agents. After decellularization, these scaffolds retain the heart's structure, fibrous ECM morphology, and vascular tree [53,184]. Using hiPSC-derived pluripotent cardiovascular progenitor cells (MCPS) that can produce cardiomyocytes, smooth muscle cells, and endothelial cells, the researchers inoculated MCPS in decellularized mouse hearts and promoted differentiation into cardiac lineage cells, finding that the decellularized scaffold itself provided a signal that promoted differentiation [185]. It gives a new idea for heart transplantation and replacement (see Table 2).

Table 2
Application of cardiovascular organoids.

Application	Model	Research contents	References
Drug screening	Human heart organoids (hCOs)	Synergistic activation of mevalonate pathway and cell cycle network by proliferative compounds	[160]
	Human ventricular like cardiac tissue strips (hvCTS) and organoid chambers (hvCOC)	Pharmacological responses of 25 cardioactive compounds covering various drug classes	[161]
	Aortic smooth muscle chip	Potential drugs such as digoxin and 2-methoxyestradiol were found for the treatment of thoracic aortic aneurysms	[152]
	Microfluidic technology-related microphysiological aorta model in vitro	Detect the drug action of metformin in the system	[153]
	Human capillary organoids	Human recombinant soluble ACE2 treatment can protect these vascular organoids from SARS-CoV-2 infection	[162]
Drug toxicity detection	A 3D printer creates a stentless three-dimensional (3D) tubular heart structure	Contraction and cell viability of cardiac structures were measured after medication	[163]
	Cardiac organoids	The co-administration of metformin was found to partially reverse the arrhythmia caused by the chemotherapist mitoxantrone	[166]
	3D heart tissue	Human ether-relevant gene (HERG) channel blockers with a high risk of arrhythmia were found to cause prolonged contractile-relaxation duration and arrhythmia-like waveforms	[167]
	Human heart microtissue (MT) model microfluidic organ chip	Lays the foundation for studying how organ-specific EC barriers respond to drugs or inflammatory stimuli	[105]
Testing the effectiveness of gene therapy	An organ chip that mimics cardiac tissue	The study found that the BTHS cardiomyopathy phenotype is easily reversed by reintroducing wild-type (WT) TAZ or inhibiting excess reactive oxygen species (ROS) produced by BTHS mitochondria	[168]
	hiPSC self-organizing heart organoids	Patient-derived induced CM from RyR2-H29D mutations leading to polymorphic	[169,170]

(*continued on next page*)

4. Summary and prospect

4.1. Research progress of cardiovascular organoids

Over the past decade, cardiovascular diseases and their organoids have been extensively studied. Induced differentiation by ESC, PSC, ASC, etc. More and more organoids are constructed through technologies such as tissue engineering, 3D printing, self-assembly, and microfluidic organ chips. There is also the combination of different technologies to generate more in line with the needs of organoids, and their function is becoming closer to the human body. The four construction schemes of cardiovascular organoids have different application scenarios respectively. The tissue engineering construction of organoids is mainly used for treating cardiovascular diseases and constructing disease-modeling organoids. 3D printing construction is primarily used to print organoids with high complexity and morphological requirements. Self-assembly construction is easy to operate and has high universality. The organoids constructed by microfluidic chips are mainly used for drug research and development and regulation of physiological and pathological factors. Organoids are widely used in vitro modeling of cardiomyopathy, myocardial infarction, cardiovascular injury, congenital heart disease, arrhythmia, and vascular and valvular diseases. It provides an essential platform for disease mechanism research, drug discovery and toxicity detection, cardiovascular development research, and disease treatment program research.

4.2. Cardiovascular problems existing in the development of class organs

While the development of cardiovascular organoids is speedy, there is still a long way to go for the research and development of organoids, such as the high cost of organoid modeling, the size of organoids, the vascularization of organoids, and the lack of microenvironment cells such as immune cells and stromal cells [186]. Therefore, selecting

Table 2 (*continued*)

Application	Model	Research contents	References
		ventricular tachycardia was modified by gene editing, and the organoid got right	
Cardiovascular physiology and development research	hiPSC self-organizing heart organoids	The process of early heart development was reconstructed, and Wnt-BMP was related to the formation of the heart cavity	[85]
	Self-organizing human multilineage organoids	The effects of epicardial cells on the proliferation and maturation of cardiomyocytes were summarized	[173]
	hiPSC self-organizing heart organoids	Activating beta-catenin and YAP1 can promote heart maturation	[174]
	Self-assembly developing heart	*PS*-NPs cause cytotoxicity of hESCs and hinder cardiac differentiation	[177]
Treatment of disease	Tissue engineered heart patch	The cardiac patch was designed to treat myocardial ischemia	[178]
	Tissue engineered cardiac organoid	Cardiac organoids designed with electrically conductive silicon nanowires (e-SiNW) can effectively repair heart tissue	[179]
	3D eX In Vivo Muscle Engineering Tissue (X-MET)	Mechanical stimulation was found to trigger functional remodeling of the 3D skeletal muscle system into myocardium-like structures	[180]
	Extracellular matrix scaffolds for acellular organisms	Studies have shown that biological stent therapy is associated with improved myocardial perfusion, reduced myocardial scar load, and reverse structural remodeling	[181]
	Decellularized scaffold	The decellularized scaffold itself provides a signal that promotes differentiation	[185]

The table summarizes the application of the cardiovascular organoid model mentioned in this paper in research, including drug screening, drug toxicity detection, effectiveness detection of gene therapy, cardiovascular physiology and development research, and disease treatment. It also includes the constructed model and the research content.

materials as scaffolds for organoids is a problem, and the influence of various substrates on organoids also needs to be explored. In addition, although there are multiple methods to improve the maturity of organoids, it is difficult to reach the level of adult heart tissue. So, we need to continue to study the organoid maturation program.

In the process of tissue engineering organoids, the material's rigidity reduces the flexibility of the cell tissue, and tissue shortening during contraction is hindered [187]. Another problem is that the structural complexity of organs in vivo is challenging to model, such as CM from atrial and ventricular myocardium with profound physiological differences, such as genetic characteristics [188], calcium processing [189], electrophysiology, and structural and functional protein expression [188]. The four heart chambers, valves, and inflow/outflow tract, for example, cannot be faithfully reproduced with these models [190]. Therefore, there is a need for better solutions to address the size of organoids and the types of tissues and cells they comprise. Perhaps through the combination of biomaterials, nanotechnology, bioengineering, and other advanced solutions [191], organoids can be made closer to the cellular composition of the human heart, so that the organoids can further simulate the human heart. The role of cardiomyocyte maturation from pluripotent stem cells and for co-culture, namely cardiac fibroblasts or endothelial cells, needs further study [192]. Furthermore, the construction of organoids necessitates induced differentiation and multiple operational steps. There is a lack of standardized schemes or guidelines [186], which may cause noise affecting the repeatability of the experiment, resulting in certain problems in the repeatability of the experiment [193].

Although there is a lot of research on organoids at present, it mostly focuses on basic research, and there are few achievements in the treatment of various diseases or the research of new drugs in the clinic. This may be due to the current drug development cycle being long and the success rate being low. Therefore, more time and money need to be invested in the development of therapies and new drugs using cardiovascular organoids.

4.3. Potential future developments in cardiovascular organoids

To address the existing limitations, future organoid research may focus more on the optimization of the construction scheme of organoids and the scale and repeatability of organoids so that their structure and function are closer to the human cardiovascular system, and they can be better used in scientific research. Scaffolds used in tissue engineering need further to shed light on their role in organoid construction, explaining their specific effects on organoids that mimic the human heart as distinct from the cardiac matrix, especially for some organoids constructed using extracellular matrix from other animals. In addition, through further research on heart development, key pathways and targets for regulating heart maturation can be found, and drugs or regulatory systems that promote organoid maturation can be designed, including cytokines, physical influences, or gene regulation. They are bringing the organoids closer to the adult heart.

As for the size of organoids, the key to solving this problem lies in the vascularization of organoids and the abundance of organoid tissues and cells. In order to form a normal function of large cardiac organoids, in addition to ensuring good blood supply and the main cells that make up the heart muscle, there also need to be enough fibroblasts, endocrine cells, nerve cells, immune cells, endothelial cells, and so on. In this way, large-scale cardiac organoids can survive and function normally so that heart replacement can be achieved in the future. For the repeatability of the experiment, the differences can be eliminated by ensuring the consistency of the cell source, the accuracy of the experimental operation, and the partial machine replacement. More importantly, the current organoid research is uneven, so it is necessary to summarize a complete and detailed operating process and provide a systematic evaluation system such as Fig. 2. In this way, the reproducibility of the model in organoid research can be further ensured.

As organoid research has progressed, multi-organ models have also gradually begun, such as linking multiple tissues through vascular perfusion to influence each tissue (increasing its fidelity by cell continuation in a tissue-like environment in the body) as well as the entire multi-organ model (by providing system components such as metabolism, immunity, and clearing needed for physiological studies) [194]. One team reported multi-organ tissue-on-a-chip systems in which mature human heart, liver, bone, and skin tissue niches are connected by

circulating blood vessel flow to allow re-summarization of interdependent organ functions [195]. The interconnected tissues maintained their molecular, structural, and functional phenotypes in 4 weeks of culture, summarized the pharmacokinetic and pharmacodynamic characteristics of human doxorubicin, allowed the identification of early miRNA biomarkers of cardiotoxicity, and increased the predictive value of clinically observed miRNA responses relative to cultured tissues alone and interconnected tissues in the absence of an endothelial barrier [195]. It can be seen that each organ will undergo extraordinary changes after a multi-organ connection. The multi-organ system can better reflect the changes in the human body, especially the endocrine system and the immune system, on the influence of each organ. Organoid models of multi-organ systems may be studied more in the future. Of course, the application of organoids will also be more expanded with the development of organoids.

Overall, the research on cardiovascular organoids is still in the early stages, and more uniquely designed regulatory schemes are needed to improve the construction of cardiovascular organoids and multi-organ system organoids further, which has great room for development and progress.

Ethics approval and consent to participate

Not applicable.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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CRediT authorship contribution statement

Xingchao Du: Writing – review & editing, Writing – original draft. **Hao Jia:** Supervision. **Yuan Chang:** Conceptualization. **Yiqi Zhao:** Validation. **Jiangping Song:** Project administration, Funding acquisition.

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