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REVIEW ARTICLE

Modelling Cardiovascular Diseases Using Human Microphysiological Systems

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Abstract

Despite pharmacological-, technological- and medical- advances, cardiovascular diseases (CVDs) remain the main cause of death and disability in the world. This underscores the urgent need to better understand the early stages of these diseases for effective prevention, as well as to develop patient-specific pharmacological approaches (personalized medicine) and novel therapies. Traditional in vitro and in vivo models often fail to accurately mimic human physiology, limiting their translational potential. In this context, microphysiological systems (MPS) have emerged as advanced in vitro platforms that integrate key physiological features of cardiovascular tissues. This review summarizes the state-of-the-art advancements in in vitro models for studying CVDs, with a particular focus on emerging 3D cardiac and vascular models. These models serve as essential tools for disease modelling, drug development, and toxicity testing. Key parameters to consider when developing cardiovascular MPS are highlighted, along with a discussion of the advantages and challenges associated with each model system.

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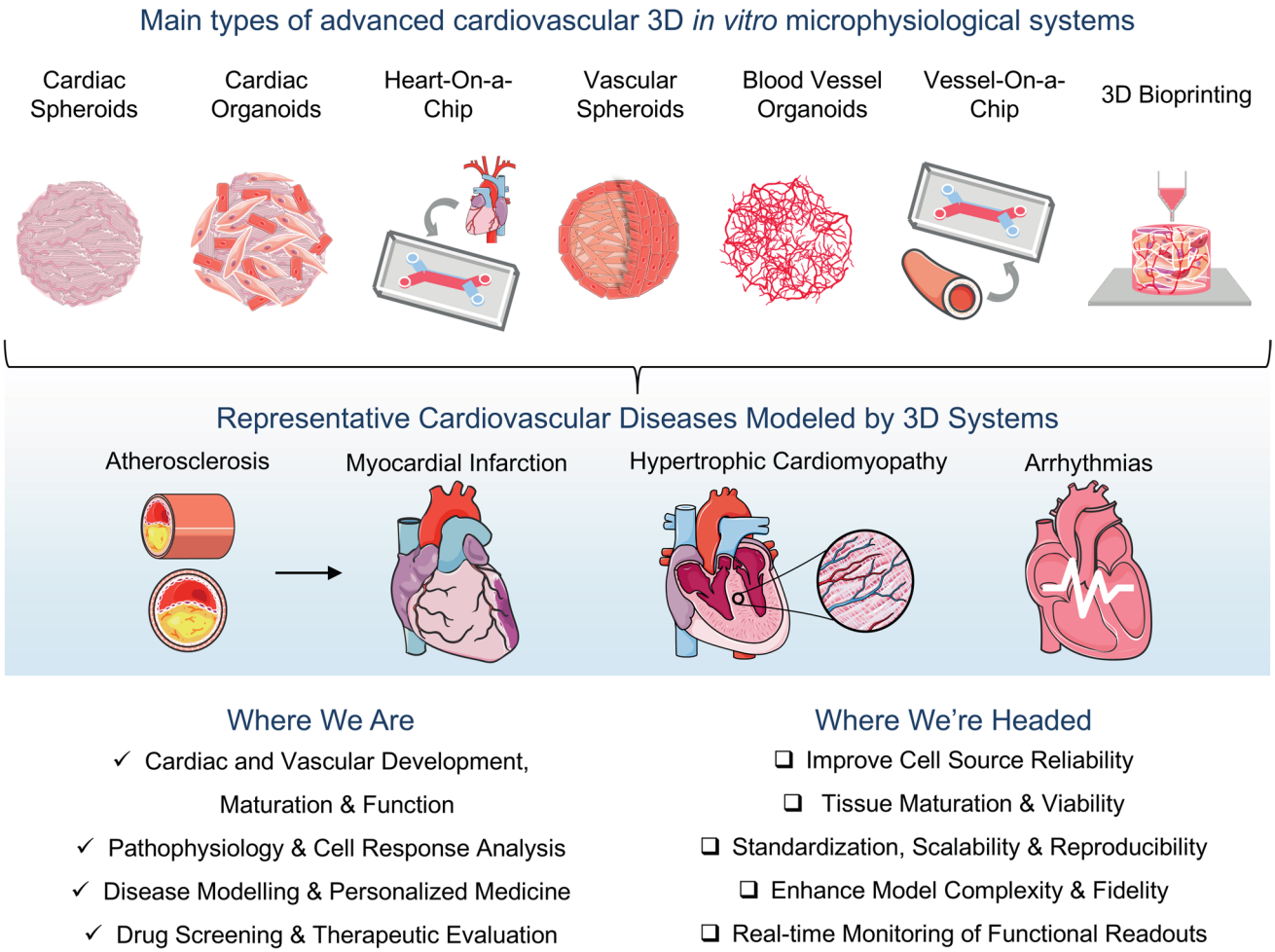
Graphical Abstract



Keywords Cardiovascular disease modelling · 3D cell culture · Spheroids · Organoids · Organ-on-chip · Bioprinting · Model development parameters

1 Introduction

Cardiovascular diseases (CVDs) encompass a range of heart and blood vessel disorders, being the leading cause of mortality and morbidity worldwide. According to the World Health Organization (WHO), CVDs are responsible for an estimated 17.9 million deaths annually, which account for 32% of global mortality (Fig. 1) [1–3]. Beyond their significant health impact, CVDs pose an enormous economic burden. Reports from the American Heart Association and the European Society of Cardiology, indicate that clinical CVD conditions resulted in $422.3 billion and €281 billion healthcare costs in the United States of America (USA; 2020) and European Union (EU; 2021) [4–6]. Despite the substantial advancement in pharmacological and device-based therapeutic options, effectively treating and preventing cardiovascular conditions, such as heart failure, remains a significant challenge [2, 7].

The identification of precise molecular targets and the development of novel effective therapies is further advanced by the growing knowledge of CVD aetiology, molecular mechanisms, and progression. Bridging fundamental and translational cardiovascular research has historically relied on studies using ex-vivo and in vivo animal models, as well as in vitro models [1, 2]. Ex vivo models can offer a close approximation of the natural cardiac structure and are suitable for studying disease conditions involving ischemia or rhythm disorders. However, they are often limited by their short-term applicability as the isolated hearts undergo constant deterioration [8–11]. In vivo models, such as genetically modified rodent animal models, have been a foundational element in preclinical research, enabling research in a living system and offering detailed

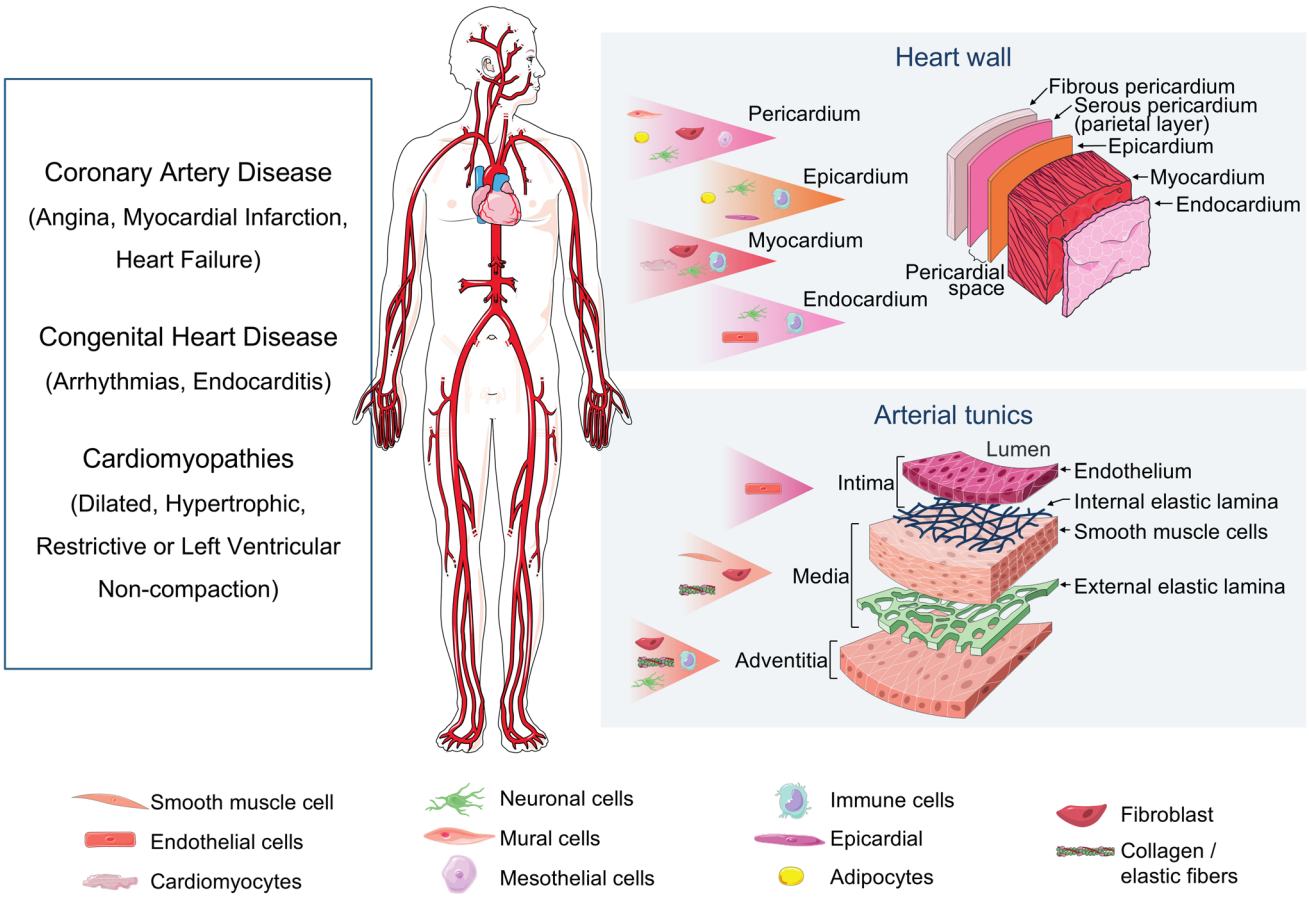


Fig. 1 Cardiovascular diseases encompass a wide range of conditions—including coronary artery disease, congenital heart defects, and cardiomyopathies—that disrupt the normal structure and function of the heart and vascular system. Both the heart and arteries are complex, multi-layered organs composed of diverse cell types that work together to maintain cardiovascular homeostasis. The heart consists of the endocardium, myocardium, epicardium, and pericardium, each contributing to electrical conduction, contractility, and mechanical protection. Similarly, arteries are organized into three concentric layers: the intima, media, and adventitia. While there is some overlap in cellular composition, including the presence of endothelial cells, fibroblasts, and immune cells, each organ and layer is characterized by unique features. For instance, cardiomyocytes compose most of the myocardium and are essential for contraction, whereas vascular smooth muscle cells in the arterial media regulate vessel tone and blood pressure. Disruption in any of these layers can initiate or exacerbate disease. A detailed understanding of this structural and cellular complexity is fundamental to unravelling the pathophysiology of cardiovascular disorders. Adapted from Smart Servier Medical Art, under a Creative Commons Attribution 3.0 Unported license [3]

insights into disease mechanisms [1, 2, 12]. Nevertheless, these models often fail to fully recapitulate the human disease phenotype and progression of human CVDs, making the direct translation to human physiology challenging [13]. Given these limitations, Göttingen Minipigs have emerged as an alternative animal model due to their similarities to humans in terms of anatomy, physiology and biochemistry. While they demonstrate promise for translational research, their use is constrained by high cost compared to rodent models [14–17]. Furthermore, the reliance on animal models raises increasing ethical concerns and reinforces the need for alternative approaches [1, 2, 12] Two-dimensional (2D) cell culture platforms have long played the pivotal role in reducing the need for animal models. These platforms facilitate studies on biophysical and biomolecular mechanisms of cardiac and vascular tissues, primarily using cardiomyocytes (CMs) and endothelial cells (ECs) to represent each tissue, respectively. Over the years, novel techniques, such as co-cultures and incorporation of extracellular matrix (ECM) components into the cultures, have been developed to improve the physiological relevance of 2D models, enhancing their potential for disease modelling, drug target identification and toxicity studies. However, these in vitro systems feature some limitations including the inability to replicate the structural and cellular complexity of native cardiovascular tissue. These limitations stem from the absence of a 3D architecture that accurately represents essential cell–cell and cell-ECM interactions, and absence of essential cell types such as epicardial, endocardial, mural and immune cells [18, 19]. Additionally, they fail to mimic key physiological factors such as nutrient delivery, gas exchange, and drug exposure gradients [18–21]. Finally,

the 2D environment often induces partially artificial phenotypes, further limiting their physiological relevance [22].

To overcome these challenges, there has been a growing focus on developing 3D in vitro models that better model cardiovascular physiology, pathophysiology and developmental processes [19, 23, 24]. This need has driven the emergence of microphysiological systems (MPS), advanced platforms designed to model human organ and tissue functions in health and disease, with unprecedented physiological relevance and precision [25, 26]. According to the U.S. Food and Drug Administration (FDA), MPS are defined as "microscale cell culture platforms for in vitro modelling of functional features of a specific tissue or organ of human or animal origin by exposing cells to a microenvironment that mimics the physiological aspects important for their function or pathophysiological condition" [27]. These systems incorporate key elements of native tissues, including 3D structures, microenvironmental features, controlled cell–cell and cell–matrix interactions, and in situ monitoring of disease initiation, progression, and drug responses [28, 29]. Additionally, MPS often utilize human-derived cells reducing the disparities between model and patient responses, making them more representative than traditional models [26, 30].

Compared to conventional in vitro models, MPS enhance cellular differentiation, growth, migration, and tissue organisation. Their ability to replicate human organ morphology and (patho)physiology makes them particularly promising for modelling complex and dynamic systems like the cardiovascular system [19, 26, 31, 32]. In cardiovascular research, these models enable the study of early pathological events and disease progression [2, 12, 25, 33–40]. Furthermore, they hold significant promise for drug development and toxicity screening, allowing for assessment of both structural and functional abnormalities of cardiac and vascular cells. By reducing the occurrence of false positive results—common in animal-specific findings–, MPS can improve clinical trial success rates, ultimately lowering drug development costs and timelines [19, 39, 41, 42]. Overall, MPS represent a transformative tool in CVD research providing new insights into different (patho)-physiological and -biological mechanisms. They are tailored to specific tissues and experimental needs, resulting in easier manipulation and maintenance compared to traditional models [1, 26, 32, 39]. Nonetheless, advancing the complexity and clinical relevance of cardiovascular MPS still depends on key several factors, such as sourcing appropriate cell types, achieving tissue maturation and vascularization, integrating immune and neuronal components, developing standardized and reliable real-time monitoring platforms, and ensuring reproducibility across laboratories [1, 24, 26, 39].

This review provides a comprehensive overview of the latest advancements in in vitro CVD research. We highlight groundbreaking studies involving cardiac and vascular 3D models, which have been utilized for disease modelling, (personalized) drug development, and toxicity testing [39, 41]. Additionally, we discuss the critical parameters for the development of different types of cardiovascular MPS along with their current potential and future perspectives.

2 Emerging in vitro MPS models in Cardiovascular Research

The human heart and vasculature form a complex circulatory network that maintain the organism homeostasis by delivering nutrients and oxygen to all cells in the body [43, 44]. Their development and function are influenced by physical (matrix micro-/nanostructures and stiffness), mechanical (fluidic forces and mechanical stretch) and biochemical (growth factors and cytokines) factors [45]. Additionally, dynamic cardiovascular processes, such as muscle contraction, ECM remodelling, and vasoregulation, are governed by intra- and inter-tissue cell interactions [43, 46]. These interactions involve a diverse array of cell types that contribute to the structure and function of the heart—comprising atrial and ventricular cardiomyocytes (CMs), fibroblasts, endothelial cells (ECs), mural cells, immune cells, and neurons – and of the vasculature, which primarily consists of ECs and mural cells such as vascular smooth muscle cells (VSMCs) and pericytes (further details on the cellular composition and tissue structures can be found in Fig. 1) [46, 47].

Accurately replicating the primary functions of the cardiovascular system in vitro, requires MPS to integrate all these key features, while maintaining ease of cell culture and analysis [45, 48–51]. Achieving this balance remains a significant challenge. Nevertheless, cross-disciplinary efforts have driven remarkable progress, resulting in innovative 3D multicellular platforms, such as spheroids, organoids, organ- or tissue-on-a-chip systems, and 3D bioprinting technologies, which will be further discussed in this section—an overview of their foundational features can be observed in Table 1 [24, 25, 35, 52]. Interestingly, these systems hold a great promise for studying human cardiac developmental biology, investigating CVD initiation and progression, identifying novel therapeutic targets, and evaluating the safety and efficiency of drugs [1, 39, 45].

3D cardiovascular tissues can be generated either through a scaffold-free or a scaffold-based approach, depending on the model's complexity, intended application, and required level of physiological control—such as electromechanical properties or biochemical gradient manipulation [1, 25, 39,

Table 1 Comparative analysis of the different microphysiological systems

3D models	Key features	Constraints	Main applications	References
Spheroids	Scaffold-free Self-assembly Simple Cost-effective Ease of automation Functional and phenotypic relevance	Limited tissue maturation/complexity Lack hierarchical tissue organization Restricted size → Necrotic core formation Lack of vascularization Absence of mechanical cues Batch-to-batch variability Short-term viability	Basic physiology & cell–cell interaction studies High-throughput functional & drug screening Personalized medicine Disease modelling (only specific pathological features)	[1, 19, 39, 45, 53–55, 66, 67, 74, 77, 82, 83, 91, 100–103]
Organoids	Scaffold-supported tissue development Self-organization Structural and cellular complexity Recapitulation of developmental processes	Incomplete representation of mature tissues Vascular and mechanical immaturity Low reproducibility and scalability Batch-to-batch variability: size, shape, cellular composition Short-term viability Protocol complexity	Modelling organ development and disease Multicellular interactions studies Mechanistic & pharmacological testing Patient-specific disease modelling	[1, 18, 24, 39, 53, 54, 74, 122–124, 126–129, 141, 154, 159]
Organ-on-chip	Scaffold-based Physiological relevance and complexity Controlled microenvironment Functional readouts and biosensing Modularity External stimuli	Limited tissue maturation Complex fabrication and technical demands Limited inter-lab standardization and reproducibility Material constraints High cost and low throughput	Physiological and mechanistic studies Real-time functional analysis Disease modelling Drug discovery and testing Precision and personalized medicine	[1, 2, 24, 26, 32, 35, 36, 39, 48, 109, 157, 159–170, 189, 190]
3D Bioprinting	High structural complexity Spatial control Customization Multi-material and multicellular integration Functional readouts	Bioink limitations Technical and manufacturing complexity High cost Functional immaturity Limite vascular integration and perfusion Low scalability and throughput	Tissue engineering Disease modelling and patient-specificity Regenerative potential	[36, 45, 201–220]

53, 54]. Scaffold-free methods rely on the cells' natural ability to self-assemble into 3D cell spherical aggregates and to produce their own extracellular matrix (ECM), originating structures, like spheroids, lacking external matrices. These systems are particularly suited for basic cardiovascular functional studies, where intrinsic cell signalling is of interest, or for drug diffusion and toxicity screening, where the model simplicity, reproducibility, and ability to mimic key aspects of the in vivo microenvironment are advantageous. However, their limited structural complexity and lack of mechanical inputs make them less suitable for capturing disease-specific features that rely on cell–matrix interactions, such as impaired ECM remodelling, a hallmark of many CVDs [1, 39, 53–55].

Conversely, scaffold-based methods use hydrogels or other materials—whether naturally-derived or synthetically engineered—to mimic the cell-surrounding ECM, creating a structured microenvironment that supports spatial organization, nutrient gradient formation, and structural development. Organoids are cultured within biological ECM-derived hydrogels (*e.g.*, Matrigel), which support early morphogenesis and promote tissue maturation, while organ-on-a-chip and bioprinted platforms generally employ natural or synthetic scaffolds to recreate mechanical and structural cues, important for disease-relevant tissue organisation. By allowing a controlled ECM composition and remodelling, these scaffold matrices enable MPS to better replicate the native organ's cellular complexity, architecture, and electromechanical behaviour, which are crucial not only for modelling healthy physiology but also for reproducing diverse CVD-specific pathological processes, including structural, functional, and biochemical alterations [1, 39, 53, 54, 56].

Overall, scaffold-free systems, such as spheroids, tend to be more reproducible and compatible with high-throughput screening, making them ideal for functional and pharmacological studies, while scaffold-based systems—organoids, organ-on-a-chip platforms and 3D bioprinted constructs—offer the tunability required for mechanistic disease modelling and biomechanical testing [1, 22, 39, 53–56]. Importantly, each type of MPS possesses distinctive features with ongoing advancements aimed at developing more physiologically accurate and translational cardiovascular models with significant implications for CVD research and therapeutic development [1, 25, 45, 57, 58]. Table 2 highlights the cardiovascular-specific applications of each MPS model described in this section, detailing the type of tissue replicated, system design, and the particular CVDs mimicked. For further insights into the use of specific cardiovascular MPS, namely bioengineered tissues, as building blocks for regenerative approaches please refer to the following recent studies and reviews [59–65].

3 Spheroids

Spheroids, first described in 1907 by Harrison R.G. et al., are 3D free-floating, self-assembling aggregates of cells that are formed without the use of a scaffold matrix [66, 67]. They are typically generated using the hanging drop method, where small drops of a cell suspension, composed by one or multiple proliferative cell types, are placed on a culture plate and then inverted. Gravity facilitates cell aggregation, allowing them to grow into adherent cell populations with non-uniform sizes [24, 39, 67–70]. Cell aggregation occurs through interaction with long-chain ECM fibres consisting of RGD (the tripeptide Arg-Gly-Asp) motifs that allow binding cell-surface integrin, leading to an increase in cadherin expression. Cadherin on the cell membrane binds to these on neighbouring cells, tightening intercellular connections and enabling spheroid formation [71, 72].

Although considered a reductionist model, spheroids have proven to be effective in addressing and surpassing the 2D constraints of traditional in vitro models being currently considered as simplified MPS [1, 73, 74]. While they may not fully meet the criteria of a comprehensive MPS, they facilitate 3D interactions between cells and the ECM in the absence of additional substrates, creating a physicochemical structure that closely mimics the in vivo tissue counterparts. Such features make spheroid highly valuable for mimicking the in vivo cardiovascular microenvironment and for high-throughput drug screening and testing [75–77].

3.1 Cardiac spheroids

Cardiac spheroids are 3D aggregates composed of primary adult cells and/or pluripotent stem cells-derived differentiated cells (PSCs, can be embryonic or induced pluripotent stem cells—ESCs or iPSCs). Typically, they are formed by CMs alone or in combination with stromal cells in a defined ratio, leading to the development of heart tissue-like structures, namely the myocardium [67, 74]. First findings on spontaneously beating CMs spheroids date back to late 1950s–1980s. In 1959, Moscona A. A. dissociated cells from a chick embryo, and then, through gyratory shaking in an Erlenmeyer flask, observed the reassembly of these cells into a tissue that resembled the original one [78, 79]. This discovery led to the emergence of several studies focused on the growth of dissociated spherical shaped-beating human heart cells in tissue culture [80]. However, the formal introduction and detailed characterization of cardiac spheroids in the literature only became more prominent in the last decade, according to the Pubmed index [81].

Throughout the years, studies have shown that primary CMs cultured in a 3D environment can maintain their contractile properties, making them valuable for studying

Table 2 Overview of novel 3D systems in cardiovascular disease research

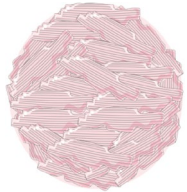
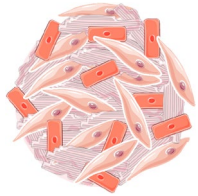
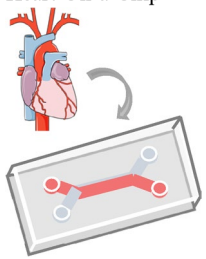
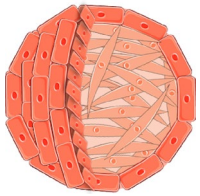
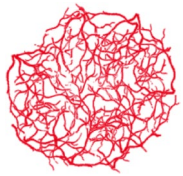
Novel 3D study systems	Tissue replicated	Design	Application in CVD research	CVDs mimicked by the model	References
Cardiac Spheroids 	Heart	Primary CMs or hPSC-derived CMs—either alone or combined with cardiac fibroblasts and human coronary artery ECs—are cultured using the hanging drop method in ratios that mimic those found in the human heart in vivo	Cardiac physiology studies High-throughput drug screening and cardiotoxicity testing Personalized medicine	Myocardial infarction Heart failure & cardiac fibrosis Cardiomyopathies Arrythmias Cardiac fibrosis Heart failure phenotypes	[19, 39, 45, 67, 74, 82–86, 88, 89, 91, 99]
Cardiac Organoids 	Heart	Derived from human stem cells, from either primary tissue, embryonic stem cells or iPSC, organoids self-organize through cell sorting and spatially restricted lineage commitment	Early heart development studies Electrophysiology and cardiac rhythms Cardiac tissue maturation Drug screening and pharmacological testing Cardiac regeneration and repair Hypoxia and ischemia studies Cardiac fibrosis and hypertrophy research Metabolic analysis Patient-specific disease models	Myocardial infarction Heart failure & ventricular remodelling Cardiomyopathies Congenital heart diseases Arrythmias Aging-associated cardiac conditions	[1, 18, 24, 26, 39, 122–124, 126, 127, 130–134, 137, 139, 140]
Heart-On-a-Chip 	Myocardium Or Whole heart	Cardiac cells (CM and non-CM hiPSCs) and associated matrices organized in a microfluidic device, with established environmental controls and analytical components	Electrophysiological studies Mechanical function and contractility assessment Ischemia–reperfusion injury studies Myocardial remodelling upon injury studies Genetic and patient-specific disease models Pharmacological testing and drug screening Cardiovascular phenomena studies Personalized medicine	Cardiomyopathies Left ventricular hypertrophy Myocardial infarction Cardiac fibrosis Arrythmias Heart failure Valve diseases	[1, 2, 32, 57, 161–168, 173, 174, 180–184, 186–188]
Vascular Spheroids 	Blood vessels	3D spherical aggregates of vascular cells, such as ECs and VSCMs, cultured using the hanging drop method	Angiogenesis studies Vascular development and homeostasis Vascular remodelling in disease conditions Extracellular matrix and fibrosis studies Inflammation and immune cell recruitment Drug screening and therapeutic targeting	Atherosclerosis Microvascular dysfunction Angiogenesis-related disorders Hypertension-induced vascular remodelling	[109–113, 115–117]
Blood Vessel Organoids 	Blood vessels	hPSC aggregates are embedded into collagen I/Matrigel substrate followed by modulation of several signalling pathways hBVOs comprise networks of ECs surrounded by pericytes, mesenchymal stem-like cells, and hematopoietic cells	Vascular development and homeostasis studies Vascular remodelling in disease conditions Vascular drug discovery Therapeutic mechanisms	Atherosclerosis Microvascular injury Coronary artery disease	[1, 128, 129, 151–154, 158]

Table 2 (continued)

Novel 3D study systems	Tissue replicated	Design	Application in CVD research	CVDs mimicked by the model	References
Vessel-On-a-Chip	Blood vessels	Versatile experimental platforms where different groups of vascular cells (e.g., ECs and SMCs) can be assembled, grown, and perfused under very defined conditions in vitro	Vascular biology studies Shear stress and hemodynamic studies Mechanisms of vascular diseases studies (IR injury, thrombosis, stenosis) Vascular cells interaction studies Drug screening and toxicity testing Personalized medicine	Atherosclerosis Coronary artery disease Angiogenesis-related disorders Hypertension	[26, 36, 189–198, 200]
3D Bioprinting	Myocardium, Blood vessels, Heart valves	Sequentially depositing biomaterials, biochemical factors, and living cells onto solid substrates to fabricate tissue constructs that mimic human tissue structure and composition	Vascularization strategies Electrophysiological studies Mechanisms of cardiac and vascular diseases studies Drug testing and toxicity screening Personalized medicine	Arrhythmias Coronary artery disease Vascular diseases Congenital heart disease Myocardial infarction Valve diseases Cardiac fibrosis Heart failure	[36, 45, 105, 106, 128, 201–204, 208, 217–220, 224, 225, 227, 229, 230, 232, 233, 235–248]

human heart biology, physiology and pharmacology. However, the limited availability of primary CMs and their functional decline over time, has led researchers to explore a minimally invasive cell source, such as the human iPSC-derived CMs (hiPSC-CMs). Spheroids composed of hiPSC-CMs, particularly derived from specific patients, offer a promising human-based, personalized cellular platform, recapitulating key aspects of the native cardiac function [19, 45, 82, 83].

Given the foetal-like phenotype typically displayed by CMs in hanging-drop generated spheroids [25, 83–86], multiple strategies have emerged to promote development and maturation, namely: (a) generating spheroids via the 96-well low attachment technique, to improve size uniformity, (b) supplementing media with biochemical components (e.g., metabolic substrates and hormones), (c) fusing multiple spheroids to form larger, compact structures with reduced core necrosis, and (d) engineering growth surfaces that more closely resemble the consistency of the human heart. By implementing such approaches, hiPSC-CM spheroids have proved effective in replicating certain mechanical and electrical features of the human adult ventricular myocardium, such as contractile amplitude and increased expression of gap-junction proteins indicating enhanced electrical coupling [83–87].

To accurately model the complexity of the native heart environment, however, additional cardiac cell types are required. Multicellular cultures comprising CMs, fibroblasts, and/or ECs, have been shown to enhance CM maturation and function, as well as to improve the structural and physiological features of the cardiac spheroids [39, 82, 86, 88]. Through strategic modulation of cell type, origin, and ratio, researchers have achieved control over heart behaviour and initiated basic vascular architecture within spheroids—enhancing their long-term viability [39, 82, 89]. Such features make these models valuable for drug screening applications, namely cardiotoxicity testing off off-target compounds (e.g., doxorubicin, an anti-cancer drug known to impair heart function), and for assessing the therapeutic potential of novel agents targeting CVD phenotypes [82, 90–95].

Furthermore, by exposing cardiac spheroids to pathological stimuli, researchers have employed them in patient-specific and general CVD testing, depending on the hiPSC source. These models have successfully recapitulated some pathological processes associated with myocardial ischemia and cardiomyopathies, like tissue stiffening and fibrosis [96–99].

Through ongoing advancements, cardiac spheroid models have demonstrated improved stability, functionality and responsiveness to physical, electrical, and pharmacological stimuli. These advances position them as a promising

high-throughput alternative to traditional methods for modelling cardiac physiology, assessing drug toxicity and efficacy [77, 83, 91, 100, 101]. Nevertheless, their broader application—particularly in complex CVD modelling—remains limited by challenges related to scalability, batch variability, long-term maturation and recreation of complex cardiac architectures, which include a fully developed vasculature [39, 74, 101, 102].

An increasing number of studies have focused on vascularized cardiac spheroids as the development of vascular network is essential for tissue survival and optimization of cellular functions. Strategies include co-culturing cardiac cells with vascular ones with or without scaffold support, supplementing media with pro-angiogenic factors, and combining cardiac spheroids with other constructs, such as vascular spheroids, microfluidic chips or bioprinted vascular units. By integrating the vasculature, not only can the size of the model be significantly increased, but the paracrine signalling provided by the endothelium also promotes tissue maturation. Collectively, these approaches aim to enhance the translational potential of cardiac spheroids by generating more physiological, customizable, and scalable models of the human heart [103–108].

3.2 Vascular Spheroids

Vascular spheroids are 3D aggregates of vascular cells, such as ECs and VSCMs, that serve as in vitro models for studying blood vessel development, function and disease [109–112]. The capillary network growth and extension from preexisting vessels, known as angiogenesis, is crucial in both physiological and pathological contexts. To study this vascular process in vitro, EC spheroids have first been introduced by Korff and Augustin in 1998 [113].

A few years later, these along with other researchers, demonstrated that vascular spheroids generated using human umbilical vein ECs (HUVECs) could be embedded in collagen gel to promote cell sprouting, providing a robust platform for analysing the angiogenic activation of ECs in vitro. Additionally, this spheroid-based angiogenesis assay has shown potential in identifying pro- and anti-angiogenic factors and assessing drug efficacy, offering a sensitive and versatile tool for screening approaches focused on human angiogenesis [110, 111].

Beyond angiogenesis, vascular spheroids are also valuable for studying interactions between ECs and other vascular-associated cell types, such as fibroblasts, pericytes, and VSMCs, which play a crucial role in vascular development, homeostasis and remodelling. By developing multicellular spheroids and exposing them to specific stimuli, researchers can investigate vascular remodelling, especially under pathological conditions—EC dysfunction and vascular calcification –, contributing to the development and progression of atherosclerosis [99, 109, 111, 112, 114]. Given that atherosclerosis is the main underlying cause of CVDs, vascular spheroids have been increasingly utilized to model this condition. Some studies have employed mono-culture spheroids of foam cells and VSMCs, key cell types for the disease initiation and progression, while others have developed more complex co-culture models incorporating myeloid cells, THP-1s (a human monocyte cell line), macrophages, dendritic and myofibroblasts, to replicate late-stage atherosclerotic lesions [115–117].

While vascular spheroids successfully recapitulate key cellular behaviours relevant to vascular function and disease progression, they fall short in replicating key structural and functional features of native blood vessels, like an organized network, physiological shear stress and perfusability, and mechanical dynamics [39, 111, 118, 119]. To overcome these limitations, recent approaches have integrated vascular spheroids with iPSC-derived cells, microfluidic platforms, and 3D bioprinting to generate more physiologically relevant, perfusable vascular models with higher architectural and functional fidelity [107, 118, 120, 121].

4 Organoids

Organoids are self-organising 3D cell aggregates derived from PSCs through an initial aggregation phase followed by directed differentiation and maturation. These structures can, to a certain extent, recapitulate the cellular heterogeneity, architecture and function of human organs in vitro [24, 122, 123]. Although the terms “organoid” and “spheroid” are often used interchangeably, they differ in structural complexity and developmental potential. Organoids are generally more advanced, exhibiting lineage-dependent spatial organization and more faithfully mimicking native organ characteristics [1, 39, 123, 124]. A key difference between the two models lies in their assembly, while spheroid typically form without external scaffolding, organoids are maintained within an ECM scaffold that provides biochemical cues and mechanical support for their development. Organoid models usually require long, complex protocols with the benefit of capturing a more diverse array of organ-specific cell types that are highly spatially organized. Remarkably, several organoid protocols include spheroids as an intermediate stage, where PSC aggregates differentiate within a 3D matrix that enables further maturation into an organoid structure [24, 52, 73, 124, 125].

Human organoids are emerging as powerful miniaturized organs which enable the study of a range of biomedical, developmental, and disease-related issues, bridging the gap between 2D cell culture methods and animal models.

In a CVD context, several types of cardiac and blood vessel organoids derived from hiPSCs have emerged in recent years for modelling heart development, disease and regeneration [1, 24]. Cardiac organoids have been developed to model the morphological and functional complexity of the heart—encompassing chamber formation (atria and/or ventricles), atrioventricular specification, electrophysiological activity and vascular structures—enabling studies of both early cardiogenesis and features of more mature cardiac phenotypes [18, 126, 127]. Additionally, blood vessel organoids provide a platform for studying key vascular processes—such as vasculogenesis, angiogenesis and vasculopathies—by faithfully replicating in vivo mechanisms [128, 129].

4.1 Cardiac Organoids

4.1.1 Models of Cardiogenesis

Cardiogenesis is a tightly regulated developmental process, that can face impairments leading to congenital heart defects—the most common non-infectious cause of death in the first year of life. To study these early cardiac events in vitro, cardiac organoids have emerged as powerful models that mimic key aspects of heart development [18, 126, 130–133].

The field of cardiac organoids began as an extension of the widely used embryoid bodies (EB)—embryo-like 3D aggregates of PSCs –, which upon exposure to growth and differentiation factors, such as bone morphogenesis protein—BMP—and activin A, could generate cardiac mesoderm and initiate primitive heart field self-organization. However, these structures lacked higher-order structural organization [134–136]. Notably, developmental cardiac organoids advance this approach by integrating additional signalling modulation that promote spatial organization and morphogenesis. Specifically, they require exogenous lineage-commitment signals, such as *Wnt* signalling pathway activation, along with paracrine cues from non-cardiac cell types—particularly from endodermal tissues –, to recapitulate in vivo-like cardiac development [134, 137].

Recent findings underscore the importance of coordinated development between mesoderm-derived cardiac tissue and adjacent endodermal lineages for successful cardiac organoid generation [134, 137, 138]. Several studies have established multilineage cardiac organoid models developed by *Wnt* pathway modulation, enabling the co-emergence of cardiac and endodermal-derived tissues, such as foregut, liver and gut. Importantly, the presence of non-cardiac lineages, improved structural and physiological maturation of the models, allowing them to successfully resemble aspects of the early native heart. Furthermore, these cardiac organoids have proven valuable in modelling congenital heart defects, by recapitulating cardiac malformations similar to those observed in transgenic mice or demonstrating relevance to diseases involving the cardiovascular system and other organs simultaneously [133, 139]. Overall, these models advance our understanding of developmental biology by demonstrating how inter-tissue signalling shapes organ morphogenesis and maturation.

4.1.2 Foetal-like Models

Despite progress in modelling early cardiogenesis, more advanced models are required to better reflect human foetal cardiac tissue at the transcriptomic, cellular, and structural levels. Recently, several groups have successfully generated cardiac chamber organoids, representing a unique development step of the heart. These models are characterized as 3D in vitro heart models that reliably recapitulate native cardiac-like architecture and incorporate multiple cardiac cell types [134].

Lewis-Israeli Y.R. et al. and Hofbauer et al. independently generated self-assembling heart organoids, by modulating key cardiogenic signalling pathways – activin, BMP, fibroblast growth factor (FGF), retinoic acid (RA), and *Wnt*. These models created a structure containing intricate internal chambers surrounded by organized multi-lineage cardiac cells (CMs, ECs, fibroblasts and epicardial cells). Notably, they recapitulated developmental processes, such as heart field formation and atrioventricular specification, and developed a complex vasculature within the myocardial tissue. Moreover, these organoids exhibited strong functional activity, making them a powerful platform for studying intrinsic mechanisms of human cardiogenesis, dissecting congenital heart defects, and advancing translational research including high-throughput pharmacological screenings [18, 26, 126].

4.1.3 Modelling Adult Cardiac Diseases

While the models mentioned above effectively capture early cardiac development and congenital defects, more advanced 3D systems are needed to replicate the complexity of adult heart tissue and associated diseases [39, 132, 140]. Recent efforts have focused on extending organoid maturation to model adult-onset cardiac diseases. For instance, Song M. et al. established a multicellular heart organoid model to replicate age-associated pathologies, such as myocardial infarction (MI) and ventricular remodelling. By subjecting these organoids to hypoxia-induced ischemia and ischemia–reperfusion (IR) injuries, they effectively modelled pathological conditions of acute MI and subsequent cardiac fibrosis [140]. Moreover, Meier A. B. et al. developed cardiac organoids that self-organize into a ventricular myocardium

and epicardium—referred to as "epicardioids"—offering a robust platform for studying heart failure-related conditions, such as congenital or stress-induced hypertrophy and fibrotic remodelling [132].

Despite these advancements, current cardiac organoids remain limited by underdeveloped vasculature, relying on passive nutrient and oxygen diffusion, the absence of key adult cell lineages (e.g., immune cells), and low reproducibility, what hinders their ability to fully emulate adult heart physiology [24, 39, 122, 141]. Like what has been described for spheroids, integrating cardiac organoids with other advanced technologies—such as microfluidic platforms ("organoid-on-a-chip" technology) and bioprinting—enhances their physiological relevance and control [142–145]. For instance, Ghosheh M. et al. combined multicellular organoid engineering with anisotropic stress conditioning, vascularization, and organ-on-a-chip technology to develop a self-paced, multi-chambered cardiac organoid. Embedded within an electro-metabolic-mechanical sensor system, this platform enabled real-time monitoring of mitochondrial dynamics and arrhythmogenic activity, highlighting its utility for modelling adult cardiac pathophysiology [127].

4.2 Blood Vessel Organoids (BVOs)

Developing in vitro blood vessel models that accurately mimic the native 3D human vascular microenvironment, is crucial for recreating a functional cardiac system, as the vasculature sustains continuous heart activity [36, 146, 147]. BVOs incorporate multiple cell types to mimic complex cellular interactions, including EC network and surrounding supportive tissues, closely resembling in vivo vascular responses. These models enable controlled in vitro investigation of vascular mechanisms, as they accurately reproduce critical in vivo processes like vasculogenesis, angiogenesis, vasculopathies, and pathological vessel remodelling [128, 129, 148]. BVOs have already been applied to study severe microvascular endothelial injury, in diabetes and COVID-19 patients, as well as atherosclerosis [1, 128, 149–152].

The first protocol described for the development of BVOs was established by Wimmer et al. in 2019. These organoids were generated from hPSCs by embedding the cell aggregates into collagen I/Matrigel substrate and sequentially modulating vascular endothelial growth factor (VEGF), BMP, and FGF signalling pathways. The resulting BVOs exhibited key morphological, functional, and molecular features of human microvasculature, forming EC networks surrounded by pericytes, mesenchymal stem-like cells, and hematopoietic cells. Although these organoids could develop a stable, perfusable vascular tree, this feature was only observed upon transplantation into mice, which may limit their broad applicability for the study of more complex pathogenesis mechanisms in vitro. Nevertheless, they have proven efficiency in mimicking in vitro microvascular changes commonly observed in diabetic patients, allowing for the study of intricate vascular remodelling phenotypes [129, 150]. Additionally, Khan A.O. et al., demonstrated that BVOs, comprised of ECs and pericytes, recapitulate vascular damage upon cell uptake of SARS-CoV2, further highlighting their relevance in disease modelling [149].

For modelling atherosclerosis, vascular organoids, mainly generated via Wimmer's protocol, have been employed to investigate specific pathological features, namely vascular permeability, endothelial dysfunction, altered vascular mural cell coverage, and angiogenic sprouting [128, 153, 154]. However, their potential for studying more complex processes, like excessive proliferation of VSMCs, macrophage infiltration, or plaque formation, is often limited by the misrepresentation of the tunica media and adventitia of the native vessels. Additionally, their inability to generate larger vessels restricts their structural complexity and prevents formation of perfusable arteriole- or venule-like structures in vitro [128, 129, 154]. To overcome these drawbacks several strategies, beyond transplanting the model into a live organism, have been proposed. These include integrating the vascular organoids within microfluidic systems, and converging stem cells technologies and bioengineering methods to generate more complex BVOs (tissue engineered blood vessels—TEBVs) [115, 155–157]. While most of these alternatives focus on recapitulating early stages of plaque formation, Kong D. et al. have been the first to establish a BVO-based in vitro model for atherosclerosis that can be utilized for the study of the pre-rupture disease microenvironment, where complex interactions occur between vasculature and immune cells. By including pump-induced perfusion, this model has demonstrated its potential for recreating a more accurate vascular environment, as well as in advancing drug discovery and elucidating therapeutic mechanisms [158].

5 Organ-on-a-Chip

Organ-on-a-chip models are microfluidic devices designed to replicate the key structural and functional features of specific tissues or organs enabling in-depth exploration of complex cell and molecular biology questions [24, 159]. These models address critical limitations of spheroid/organoid cultures by incorporating controlled microenvironments that overcome passive nutrient and oxygen diffusion hurdles, which hinder core maturation, as the size increases, and lead to necrotic core formation [24, 32, 36, 48, 160].

Typically fabricated from transparent, flexible polymers such as polydimethylsiloxane (PDMS), these devices contain hollow microchannels where multiple cell types can be grown in a 3D matrix, simulating native tissue environments through physiologically relevant biomechanical cues, such as fluid flow [161]. The use of primary- or stem cell-derived cell populations supports the formation of organ-specific structures with intrinsic functions [32]. Moreover, the microfluidic design enables precise regulation of cell–cell/cell–matrix interactions and mechanical/electrical/biochemical stimuli, allowing for the study of complex signalling pathways, disease mechanisms, and drug effects with enhanced accuracy [161–165].

Microfluidic-based technologies offer several advantages, including precise environmental control (*e.g.,* temperature, pH, nutrient supply, waste removal), physiologically relevant microscale features, laminar flow mimicking blood perfusion, and low sample working volumes. The dynamic manipulation of physicochemical properties and extracellular parameters within the MPS creates more in vivo-like (patho)physiological microenvironments, enhancing their potential for pharmaceuticals' testing. In addition, biosensor integration enables real-time monitoring of culture conditions and functional parameters. Depending on the application, those platforms can function as standalone fluidically isolated systems or be linked through fluidic circuits to simulate the interactions of interconnected tissues, further expanding their potential for multi-organ disease modelling and drug screening [1, 32, 166–168].

Despite all these advantageous features, organ-on-a-chip technologies present challenges, including technical complexity, high-production costs, and limited tissue maturity that may not fully recapitulate adult phenotypes. Additionally, reproducibility among different laboratories remains a concern due to the emerging nature of these platforms and the absence of standardized protocols [39, 157, 165, 169, 170].

In this section of the review, we highlight recent advancements in the cardiovascular field, focusing on heart- and blood vessel-on-a-chip platforms. These models offer powerful tools for studying cardiac physiology, vascular function, and disease mechanisms in a controlled, human-relevant context.

5.1 Heart-on-a-Chip (HoC)

HoC models leveraging microfluidics, cardiac tissue engineering, microfabrication, and integrated electronics, are integral components to develop human-relevant models. These models are used to study cardiac function, CVDs, drug toxicity, and therapeutic efficacy [2, 165, 169]. Initially developed as simple, CM-based platforms, HoC models have evolved into more complex multicellular systems that include stromal and endothelial cells [171–173]. Along with the integration of microchambers with controlled fluid flow, these advancements enable more accurate replication of the cardiac microenvironment, allowing for detailed analysis of cell behaviour and organ-level dynamics [2, 165, 169].

The conceptualization of HoC systems begins with clearly defined research objectives. Depending on their primary function, these models have been traditionally classified as either electrical or mechanical stimulation-based systems [2, 165, 169, 174]. Electrical systems, such as Biowire I and microelectrode array (MEA)-based chips, have been pivotal for the study of electrophysiological properties [175–177]. While mechanical systems, such as muscular thin film (MTF) devices, have enabled the quantitative assessment of contractility and structural properties, like cellular architecture and tissue-generated stress [172, 178].

Recent advancements have transcended this binary classification with next-generation HoC platforms now integrating electrical and mechanical cues with biochemical gradients. This multifactorial approach enables a more faithful replication of the heart's native electromechanical function and its response to physiological and pathological stimuli in vitro [157, 165, 169, 179]. For instance, the Biowire II platform employs continuous electrical stimulation via carbon electrodes combined with passive mechanical loading from flexible polymer wires [180, 181]. Due to such complementary stimulation cues, the multicellular iPSC-derived cardiac tissues embedded in 3D hydrogels within this system exhibit enhanced maturation and adult-like phenotypes. By strategically modulating the iPSC source (healthy or patient-derived), cell composition, tissue architecture, and external cues, researchers have used such platform to model cardiomyopathies [180], left ventricular hypertrophy [181], calcific aortic valve disease [182], myocardial ischemic injury [183], and fibrosis with high fidelity [184].

As HoC platforms become increasingly physiologically relevant, there is a growing integration of multimodal sensors that enable non-invasive, real-time monitoring of cardiac tissue behaviour and environmental conditions [165, 169, 185]. The integration of such sensing modalities is essential for maintaining controlled and reproducible culture conditions within HoC systems while allowing longitudinal data acquisition with clinical relevance [157, 185]. Nevertheless, miniaturizing sensors to match the microscale of organ-on-a-chip platforms remains a challenge, as smaller sizes can reduce sensitivity and signal resolution, making it difficult to detect subtle changes in force or electrophysiological activity. Additionally, material compatibility issues may affect sensor performance and compromise tissue viability within the chip [19, 45, 159].

CVD modelling has nonetheless benefited from these integrated approaches. For example, an arrhythmogenic right ventricular cardiomyopathy (ARVC)-on-a-chip system—constructed using patient-derived iPSC cardiomyocytes harbouring DSG2 mutations—incorporated both direct and indirect contraction force measurements. By video-tracking the deflection of the PDMS flexible pillars anchoring the engineered cardiac tissues (direct), and performing calcium imaging to analyse the intracellular calcium dynamics (indirect), the researchers successfully captured key ARVC phenotypes, including arrhythmic beating, reduced force generation, and abnormal calcium handling [186]. Similar systems, like cardiac fibrosis- and arrhythmia-on chip, developed by Mastikhina O. et al. and William K. et al., respectively, also incorporates flexible rods to assess force dynamics during tissue contraction, while electrode integration in arrhythmic models enables detection of conduction abnormalities associated with electrical remodelling [187, 188].

Briefly, these studies support that continued innovation in microengineering and sensor integration will be key to further improving the fidelity, scalability, and clinical relevance of HoC platforms in cardiovascular research [186–188].

5.2 Vessel-on-a-Chip (VoC)

VoC models are advanced 3D microfluidic platforms designed to replicate the native microenvironment and architecture of the human vasculature [109, 164]. By integrating fluid flow and external regulation systems (e.g., pumps), these models enable dynamic culture conditions that closely mimic vascular morphology, function, and interactions [35, 109]. Controlled mechanical stimulation provides insight into shear stress effects on vascular biology, while real-time imaging allows for continuous structural and functional assessment [26, 189, 190]. Additionally, VoC systems support mechanical and electrical testing, making them valuable tools for studying vascular physiology and disease mechanisms, including the progression of atherosclerosis [26, 36].

VoCs can be categorized based on their geometry which varies depending on the experimental goals. The "endothelium covering a channel" models are often used for generating larger vascular constructs or for compartmentalizing different cell types, while the microvascular networks (μVNs) are designed to mimic the microvasculature. The shape of the vascular structures in the first category depends on the channel's configuration within the device [26]. One type of model in this group uses multiple layers of channels separated by polymer-based semi-permeable membranes coated with ECM proteins like fibronectin. ECs and other cell types, such as VSMCs, are cultured in separate channels, interacting through the flexible porous membrane, which mimics the internal elastic lamina. An example of this model is the hemodynamic EC–VSMC-signalling-on-a-chip which enables cell–cell interactions, crucial for the development and homeostasis of the endothelium and the basal lamina. This model has proven valuable for studying biological and mechanical components of the vessel, as well as vascular diseases such as atherosclerosis and hypertension. It also enables real-time cell imaging, hemodynamic condition control, and drug testing [26, 191, 192]. Another model in this category involves parallel channels featuring micro-pillars or guide-edges. One of the channels is filled with hydrogel or collagen to establish the ECM and ECs are injected into the adjacent channel to form a vascular structure. These models are particularly valuable for studying early atherogenic events and complications of atherosclerosis (*e.g.,* stenosis), as they allow variations in blood vessel geometry and perfusion and VSMC migration analysis [26, 193, 194]. A third type involves cylindrical blood vessels, that are created by forming a cavity within an ECM chamber using a needle, which is removed after the solidification of the hydrogel. VSMCs and ECs are then injected sequentially into the cavity to form a vascular structure. Such models closely mimic the arterial architecture, providing a foundation for integrating physiological functionality and simulating vascular disease, including early events of atherosclerosis like foam cell formation [26, 195–198].

The μVNs category focuses on creating smaller, physiologically accurate vascular systems and can also be separated in subgroups. In self-organized μVNs, ECs are typically embedded in a fibrin ECM, where they proliferate and naturally assemble into vascular networks. Addition of growth factors or co-culture with stromal cells aid the process of microvasculature generation [26, 199]. In angiogenesis μVNs, ECs are placed along the sides of a fibrin gel, and vascular sprouting is stimulated by co-cultures with fibroblasts or addition of growth factors [26, 191, 200].

Although various VoC formats have demonstrated significant progress in studying vascular biology and disease modelling, key challenges remain. Ongoing innovations in biomaterials and microfabrication are expected to enhance VoC physiological relevance and translational potential. These advancements aim to overcome challenges in replicating in vivo complexity while improving scalability and integration with other organ-on-a-chip systems.

6 3D Bioprinting Platforms

3D bioprinting has gained attention in recent years as an advanced additive manufacturing technology that efficiently enables the precise layer-by-layer deposition of biomaterials, biochemical components, and living cells onto solid substrates to create complex tissue constructs resembling human tissues in structure and composition [45, 201, 202]. This technique has revolutionized the development of MPS by mimicking with high fidelity the cellular architecture and physiological environment of human organs, although the resulting constructs often still present limited cell maturity and incomplete mimicry of native tissue functionality. It offers unprecedented potential for applications in regenerative medicine and organ replacement, as well as in vitro disease modelling [202–204].

Based on their working principles, 3D bioprinting methods are mainly categorized into inkjet bioprinting, extrusion bioprinting, and light-assisted bioprinting, each capable of fabricating various tissues and organs. Inkjet bioprinting creates 3D layer-by-layer structures by depositing bioink droplets onto a substrate. Extrusion bioprinting utilizes pneumatic pressure or mechanical force to dispense the bioink through a nozzle continuously and deposit it on a receiving layer. Light-assisted bioprinting encompasses nozzle-free approaches that use digital projection or laser technology to pattern bioinks with high precision. In laser-assisted bioprinting, pulsed laser energy is focused onto an absorption layer which then propels bioink droplets onto a substrate. In contrast, digital light processing (DLP)-based bioprinting uses patterned light to photopolymerize cell-laden hydrogels in a layer-by-layer or continuous manner [45, 205–212]. The accuracy of each method depends on the usage of specialized bioinks—viscoelastic materials with high water content, which maintain cell viability and functionality during printing –, which may contain live cells, biocompatible hydrogels, or/and other essential biologically active components [205, 211, 213–215]. Of note, achieving the right balance between printability, mechanical stability, and biological functionality of the existing bioinks constitutes a challenge particularly when it comes to long-term tissue viability [215, 216].

In the cardiovascular field, the above-described 3D bioprinting techniques have been applied for cardiac tissue engineering and vascularization strategies, both for therapeutic and research applications [36, 208, 217–220]. Inkjet and light-assisted methods are often used to create vascular and cardiac constructs/grafts, respectively, when high-resolution design and cell viability are critical [221–223]. While the extrusion-based technique is preferred to generate larger, functional tissues requiring higher cell densities, such as the myocardium [224, 225], heart chambers [226] and valves [227, 228]. This latter method has also been used to generate perfusable vasculature that can, for instance, be printed directly onto microfluidic devices in a hybrid approach advancing vascularized MPS platforms [229, 230].

As the field advances, several complementary or alternative approaches have been developed to provide a more physiologically relevant environment and mechanical support for cardiovascular tissue engineering. For instance, the Microscale Continuous Optical Printing (μCOP) and the Bioprinting-Assisted Tissue Assembly (BATA) methodologies were developed to complement traditional light-assisted and extrusion-based techniques, respectively. The μCOP is a high-speed, precise, and high-cell viability microfabrication light-based method, while BATA is a hybrid methodology that combines existing cardiovascular MPS (spheroids or organoids) with the bioprinting method to enhance structural and functional tissue complexity [106, 145, 231, 232]. On the other hand, the Freeform Reversible Embedding of Suspended Hydrogels (FRESH) bioprinting technique, developed by Hinton J. et al. and Lee A. et al., emerged as an alternative to traditional open-air printing methods [203, 233]. FRESH prevents structural collapse by depositing biological hydrogels into a thermo-reversible support bath that temporarily holds the construct during printing [203, 233, 234]. This approach enables the fabrication of complex biological structures using soft biomaterials (alginate, fibrin, collagen type I, and Matrigel) with enhanced accuracy and structural fidelity.

Overall, innovative methods like the ones introduced above have been used to generate several sophisticated 3D cardiovascular tissue scaffolds, namely uni- or multiscale perfusable vascular networks [203, 233, 235], cardiac ventricles [106, 203, 236], native myocardium [232, 237], heart valves [203, 238] and both neo-natal scale and full-size model of an adult human heart [203, 239]. Beyond tissue fabrication, 3D bioprinting holds promise for CVD modelling. By manipulating the cell ratio and composition within the bioprinted construct together with modulation of complex signalling pathways associated with fibrotic remodelling, BATA has been used to generate a transforming growth factor-β (TGF-β)-induced cardiac fibrosis model incorporating CMs, cardiac fibroblasts and ECs [240]. In another study, researchers refined the traditional extrusion-based method, by optimizing the printing pressure and speed, to bioprint patient-specific hiPSC-CMs carrying the RyR2 mutation, successfully modelling an inherited tachycardia condition and reproducing arrhythmic responses to adrenaline, mirroring the clinical phenotype [241].

Generally, by selecting the appropriate bioprinting approach and manipulating relevant biochemical and mechanical cues, researchers have developed in vitro models of other CVD subtypes. These include coronary artery

disease (mainly caused by atherosclerosis) [242–244], structural heart diseases (congenital heart disease) [245, 246], cardiac valve disease [247], acute heart conditions (MI) [218] and their aged-associated complications ultimately leading to heart failure [248]. Despite these promising studies, the translational potential of these models is still limited by functional immaturity of hiPSC-derived CM and the absence of complex vascular integration within cardiac constructs.

Finally, in recent years 3D printing of tissue-sensor platforms has also been described for cardiovascular applications. Yong J. et al. developed a sensor-integrated platform for simultaneous monitoring of multiple physiological parameters, such as force and electrical activity, with high potential for advanced CVD modelling and high-throughput drug screening. Using a single-step printing process with multiple materials, namely five distinct inks, one of which with electroconductive properties, and a porcine-heart-derived decellularized ECM hydrogel (dECM), they created an 3D human-engineered heart tissue (EHT) composed of CM and cardiac fibroblasts embedded with an electronic strain device which promoted EHT self-assembly, alignment, contractility, and maturation. The electronic sensor also allowed to monitor the functional factors of the cardiac MPS before and after cardiotoxicants exposure [249].

Altogether, while 3D bioprinting represents a powerful and highly customizable and methodology, its current application in the cardiovascular field is constrained by model immaturity, limited vascular integration, and biofabrication complexity [211, 216]. These challenges are continuously being addressed.

7 Design and Functional Components of MPS Used in CVD Research

The precise design of MPS allows for a close recapitulation of human organ systems by controlling tissue composition and architecture through a variety of molecular, structural, and physical cues [19, 45]. While no single MPS can fully replicate the features of an entire organ, current models capture key morphological and functional phenotypes relevant to specific tissues or diseases [19, 25, 250]. MPS development relies on the inclusion of clinically relevant cells, physicochemical microenvironments, and real-time monitoring to assess physiological responses [25]. For heart and vessel MPS, the essential building blocks, further described in this section, include relevant cardiac and vascular cell types, a supporting matrix, electromechanical stimulation, and functional measurements [25, 251].

7.1 Cell Sources and Origin

7.1.1 Heart

Selecting appropriate cell types is crucial for the successful development of MPS that accurately resemble the organ of interest and its functions [45]. The adult human heart is a structure that exhibits significant regional cellular heterogeneity being composed of atrial and ventricular CMs (49%), mural cells (21%), fibroblasts (16%), ECs (8%), immune cells (5%), neuronal cells, adipocytes, and a small population of resident multipotent cardiac stem/progenitor cells [46, 47].

Given the abundance and functional significance of CMs in myocardium, the majority of cardiac MPS described in the literature consist of monotypic cultures of CM from varying sources (cell lines, primary cultures or PSCs) [25, 252–254]. CMs cell lines have been disregarded as they behave differently from primary cells, not faithfully resembling the physiology and function of the desired tissue [45]. In contrast, primary cells, from human origin, not only are more tissue-representative, but also can be isolated from patients allowing the generation of patient-specific disease models important for personalized medicine and regenerative medicine approaches [19, 45]. However, as primary cells are difficult to obtain from most of the human organs, finite in passage number, and of difficult long maintenance in vitro, PSCs, which include ESCs and iPSCs, have drawn attention as promising alternatives to produce MPS since they are a potentially unlimited renewable cell source, which can be selectively differentiated into a variety of cell types [19, 45, 255, 256]. ESCs are associated with high ethical concerns given that they are sourced from the inner cell mass of the blastocyst requiring the destruction of embryos for their obtention. Conversely, iPSCs are isolated from body fluids, thus circumventing the ethical hurdle regarding cell source [257–259].

There are several studies where iPSC-derived CMs have been implemented for the generation of cardiac disease models given their accurate representation of human CMs. However, they often exhibit a more foetal-like phenotype [25, 86, 260]. To enhance CM maturation, culture protocols have been optimized by modifying media composition to shift the energy metabolism of cells, and incorporating non-CMs, such as ECs and fibroblasts, allowing bidirectional cell–cell interactions. Crosstalk between CMs and non-CMs is essential for proper growth and mechanical support of the native heart, and should, therefore, be considered for accurate designing of mature cardiac MPS [261, 262].

Beyond cell origin and developmental stage, the cell ratio and spatial distribution are also critical factors. Imbalance in cell proportions and changes in cellular arrangement may

lead to disturbances in paracrine communication culminating in pathophysiological features, which may become relevant when creating MPS for CVD modelling [24, 25, 39].

7.1.2 Vessels

Blood vessels play a critical role in maintaining cardiac homeostasis, by supplying oxygen, nutrients and signalling molecules [36, 147]. The vascular network acts as a selective barrier establishing an interface between blood and surrounding tissue, which is crucial in various (patho-) physiological processes [45, 263]. Developing vascular MPS, either for studying vascular mechanistic insights or for vascularization of cardiac in vitro models, requires the co-culture of ECs and supporting cells [25, 26]. As previously discussed, the cell source is essential for generating an MPS model, therefore HUVECs have long been used to establish perfusable vascular models due to their robustness, easy availability, and adaptability in various tissue engineering applications [264, 265]. Since cell passage number is a key factor for vasculogenesis processes, iPSCs are being used as an alternative to the limited passage rate of primary cells, providing a both patient- and tissue-specific cell source for MPS development [25, 232]. Despite EC monocultures being able to form vascular structures with lumens, supporting cells play a crucial role in promoting angiogenesis and enhancing vascular maturation. For instance, pericytes, an important sustaining cell type, are commonly implemented along with fibroblasts, VSMCs and mesenchymal stem cells (MSCs) in co-cultures with ECs to generate accurate multicellular vascular MPS [265–268].

7.1.3 Immune Components in Cardiac and Vascular MPS

The current trend in MPS development is shifting towards more clinically relevant cell types and improved in vitro models to more accurately mimic in vivo conditions. In this context, the inclusion of immune cells is increasingly being considered, as they are fundamental for both physiological regulation and inflammatory responses that contribute to the progression of cardiovascular diseases [1, 25, 269, 270].

Owing to the immune system's cellular heterogeneity and dynamic behaviour, its integration into MPS platforms has been both structurally and functionally challenging [271, 272]. Nevertheless, recent strategies have focused on incorporating immune components either through co-culture of the 3D tissue with terminally differentiated immune cells—maintained in a basal or activated state –, or via co-differentiation of immune lineages alongside cardiovascular cells during tissue development [272, 273]. These methods have been applied to cardiac and vascular organoids in a few pathophysiological studies improving the local microenvironment and the recapitulation of specific immunomodulatory properties [158, 272, 274, 275]. An alternative approach is the assembloid technique, in which immune organoids are fused with the organoid representing the target tissue [272, 276]. In vascularized heart-on-a-chip systems or vascular microfluidic devices, immune cells have been introduced either in circulation within the vascular lumen or integrated into the supporting hydrogel matrix [176, 273, 277, 278]. When it comes to bioprinted cardiovascular constructs, there are still no reports on the embedding of immune cells within the bioinks. Instead, their inclusion has been attempted upon the printing process, like shown by Enayati M. et al., who seeded macrophages onto printed cardiac patches intended for regenerative applications [279]. Overall, studies have mainly focused on incorporating innate immune cells, such as macrophages, monocytes and neutrophils, into cardiovascular MPS, while the integration of adaptive immune components, including T and B lymphocytes, remains unexplored. Recapitulating adaptive immunity, which plays a critical role in chronic cardiovascular conditions, represents a promising direction for future research in this innovative research domain [270, 280, 281].

Furthermore, indirect immunomodulatory approaches, such as exposing the models to cytokine gradients, have been used to promote immune interactions and better simulate inflammatory processes relevant to CVD states [282]. This strategy has already been successfully applied in other MPS contexts and may represent a practical short-term alternative for integrating immune function into cardiovascular platforms—helping address the challenges in mimicking the complexity of the native immune system in vitro [273, 283, 284]. In the long term, a more comprehensive solution lies in the integration of multiple organs into a single system, which would enable dynamic crosstalk and systemic interactions essential to accurately model cardiovascular pathological processes and immune responses [32, 41, 45].

8 Biomaterials and Scaffolds

The majority of MPS platforms heavily rely on material engineering to recapitulate the physical and biochemical properties of the native human tissue ECM. Biomaterials, whether natural or synthetic, play a pivotal role in facilitating cell–matrix interactions and in the overall fabrication of MPS, which provide a robust platform for studying human physiology and disease. These materials must not only provide the physical and chemical cues required by the cells but also possess suitable rheological and gelation properties for integration into engineered systems [25, 45, 285–287].

Hydrogel-based materials are central to the fabrication of cardiovascular MPS due to their high-water content,

biocompatibility, and ECM-mimicking features [25, 288, 289]. In cardiac and vascular organoid systems, natural hydrogels such as Matrigel, collagen, fibrin, and dECM are widely used to support cell adhesion and growth. Matrigel is particularly effective in promoting CM maturation and vascular sprouting due to its intrinsic properties that mimic the mechanical and chemical cues of the in vivo ECM. However, its undefined composition, batch-to-batch inconsistency, and animal-derived origin hampers their reproducibility and clinical translation [290–294]. To address these issues, synthetic hydrogels, such as PEG-based materials, have been increasingly applied for defined, tunable organoid cultures. These are often functionalized with adhesive peptides (e.g., RGD and IKVAV motifs) to support cardiac tissue assembly or vascular morphogenesis. Furthermore, bonding PEG-hydrogels with degradable crosslinkers allows for spatiotemporal control of specific biochemical cues, guiding stem cell differentiation and mimicking microenvironment remodelling [290–293, 295, 296].

In cardiovascular organ-on-a-chip platforms, natural or synthetic hydrogel components are typically used to coat microchannels and chambers fabricated from elastomeric organic materials, like polydimethylsiloxane (PDMS) and poly(methyl methacrylate) (PMMA) membranes, enabling the mimicking of physiologically relevant microenvironments [25, 157, 297]. In HoC platforms, materials such as methacrylated gelatin (GelMA) or collagen hydrogels support CM contractility and multicellular alignment, especially when combined with mechanical or electrical stimulation [175, 180, 297]. In VoC platforms, hydrogels like fibrin, collagen I, or hyaluronic acid (HA)-based gels enable the formation of perfusable endothelialized channels, allowing the modelling of pathophysiological conditions, like angiogenesis or atherosclerosis [26, 194, 298]. Notably, alternative elastomer materials for the fabrication of the main body of the chip have been described for specific applications like CVD drug testing. For instance, silk fibroin or polycaprolactone (PCL) have been applied in vascular chips to enhance barrier function and reduce absorption of small hydrophobic drugs, a known limitation of PDMS-based devices in pharmacological testing [179, 299].

In bioprinted cardiovascular MPS, hydrogels act as bioinks whose composition and viscosity are tailored to the specific printing technique. For example, inkjet bioprinting utilizes low-viscosity bioinks (e.g., gelatin or decellularized ECM-based solutions) to precisely deposit vascular cells in layered configurations [219, 222, 300]. Extrusion bioprinting uses high-viscosity formulations (e.g., composite bioinks, including collagen, alginate and/or GelMA) to fabricate functional cardiac components and branched vascular constructs with structural fidelity and perfusability [219, 224, 226, 230]. Light-assisted bioprinting allows for high-resolution patterning using visible-light crosslinkable bioinks, allowing the fabrication of complex cardiovascular tissues [221, 223, 300, 301]. Recent strategies using composite bioinks containing nanoparticles (e.g., carbon nanotubes or gold) have improved electrical conductivity and mechanical stability in cardiovascular constructs [302, 303].

Overall, selecting and engineering specific biomaterials is critical for developing functional cardiac and vascular 3D models that mimic native tissue behaviour. A summary of the information about the primary types of biomaterials used for the fabrication of the different cardiovascular MPS is available on Table 3. Additionally, the respective advantages, disadvantages and potential alternatives are also described.

9 Electromechanical Stimulation

The integration of electrical and mechanical stimulation in cardiac MPS plays a key role in promoting the maturation and functional performance of their composing cells, especially the cardiomyocytes (CMs) [25, 289, 304–307]. Electrical cues—delivered through systems such as MEAs, conductive scaffolds (e.g., nanocomposites of gold nanowires with macroporous alginate scaffolds), or bioreactors—not only improve CM maturation, but also enhance their electrophysiological properties, such as synchronized beating and cell–cell coupling. The application of electrical stimulation requires careful control of several factors such as pulse frequency, voltage amplitude, and substrate conductivity, which are dependent on the materials the compose the electrical systems and whose optimization improves cell response in distinct biomaterial MPS [308].

Meanwhile mechanical stimuli—either passive (matrix stiffness and passive stretch) and active (cyclic stretching and shear stress)—contribute to cell alignment, contractility, and sarcomere organization [309]. Passive mechanical stimulation refers to mechanical cues that are inherent to the MPS environment helping to mimic the native myocardium's stiffness and functional maturation. Thus, when developing a cardiac MPS, soft substrates within the physiological stiffness range of the native myocardium are selected to mimic the passive mechanical resistance CMs would experience in vivo. However, the stiffness of scaffolding materials changes over time as cells remodel their microenvironment, being crucial to characterize the substrate properties gradually [25, 305, 310]. Passive stretch is usually controlled by fixing the constructs between flexible anchors, commonly observed in organ-on-a-chip systems, which creates tension in the cell structures, promoting cell alignment and contractile function [25, 181, 297]. Additionally, active mechanical stimulation offers another layer

Table 3 Main materials used for the fabrication of cardiovascular MPS

Types of MPS	Biomaterials & scaffolds	Advantages	Disadvantages	Potential alternatives	References
Organoids	Natural hydrogel-based biomaterials: Matrigel, Collagen, Fibrin, dECM	Biocompatible Support cell adhesion and growth Complex signalling network	Undefined composition Batch-to-batch inconsistency Low mechanical strength Animal-derived origin	Synthetic hydrogels such as PEG-based materials: Functionalization with biological motifs—influence cell behaviour and intercellular communication Bonding with degradable crosslinkers—allow for spatiotemporal control of specific differentiation and biochemical cues, and mimic microenvironment remodelling	[24, 25, 45, 146, 285–296]
Organ-on-Chip Platforms	Natural or synthetic hydrogels (Collagen, Fibrin, GelMA, HA) + Elastomeric organic materials (PDMS, PMMA)	Excellent biocompatibility Optical transparency Gas permeability Ease of processing	PDMS (most common scaffold material): not suitable for CVD drug testing due to high absorption rate for small hydrophobic molecules	Alternative elastomeric materials: Silk fibroin or PCL membranes – enhance the structural and physiological resemblance observed in vivo	[19, 25, 26, 36, 157, 161, 175, 179, 180, 194, 297–299]
3D Bioprinting	Inkjet bioprinting: Low viscosity bioinks (Gelatin, dECM) Extrusion bioprinting: High viscosity bioinks (Collagen, Alginate, GelMA) Light-assisted bioprinting: Visible-light crosslinkable bioinks	Maintain cell viability and functionality during printing while mimicking the 3D extracellular matrix	Need for specific ink viscosity Low printing resolution Short-term tissue viability	Hybrid bioinks with the desired porosities & mechanical and chemical properties (addition of nanoparticles further improve their features)	[45, 217–219, 221–224, 226, 230, 300–303]

of complexity by applying controlled cyclic stretching or shear stress to the cells. Cyclic stretching, applied via bioreactors or electromechanical platforms, generates strain on the substrates improving the contractile stress, sarcomere orientation, and intercellular alignment, while shear stress, provided by perfusion within the MPS, enhances the CM beating intensity, mitochondrial density and sarcomere length, simulating early developmental processes [25, 310–312]. Combining electrical and mechanical cues, using for example stretchable MEAS, has synergistic effects, further driving CM maturation and developmental pathways [25, 289, 304–307, 313–316].

The microvasculature, especially the one surrounding the myocardial tissue, plays a vital role in supporting tissue function by ensuring efficient transport of oxygen, nutrients, and waste, while also mediating intercellular communication. The formation and function of the microvasculature are significantly influenced by the shear stress, ECM stiffness, and interactions with surrounding cells, so these factors should be considered when creating vascular MPS. Physiological levels of shear stress promote EC morphogenesis and sprouting, whereas excessive levels can compromise barrier integrity and activate pathological responses. Similarly, substrate stiffness modulates cell–cell interactions, with lower stiffness (1–20 kPa) favouring tight junction formation and maintaining endothelial cohesion, and higher stiffness (>30 kPa) leading to their disruption and EC dysfunction [45, 317–321].

A comprehensive summary of electromechanical stimulation parameters and their effects on cardiovascular MPS is provided in Table 4.

10 Functional Measurements

Designing cardiovascular MPS requires continuous assessment of functionality, typically achieved through sensor integration [25, 322]. Ideally, functional sensors should elucidate important biological pathways, streamline the identification of crucial cellular components in disease progression and suggest therapeutic windows in disease models by aiding in the determination of drug toxicity and efficacy [322, 323]. However, due to recent emergence of the field, few studies have integrated biosensors into cardiovascular MPS. Most studies have focused on liver-on-a-chip, the most established organ-on-a-chip system, where optical and electrochemical sensors are employed to monitor the following functional outputs: cell secretomes [324, 325], biochemical parameters (*e.g.,* pH and oxygen levels, etc.) [326, 327], cell signalling [328], drug response and behaviour [329].

In cardiac MPS, especially heart-on-a-chip platforms with dynamic flow conditions, functional readouts must capture the entire cardiomyocyte contraction cycle. This involves monitoring electrophysiological parameters such as membrane potential and intracellular calcium dynamics, as well as mechanical properties like contractility. In addition, biochemical measurements include temperature, pH, partial oxygen pressure, glucose and lactate levels, as well as cardiac biomarkers (e.g., troponin T and creatine kinase-MB), which are essential indicators of physiological stability and cardiac function [25, 322].

Contractility and electrophysiological properties analysis are performed in situ using highly sensitive sensors that offer real-time, high-resolution insights into the system's behaviour [25, 185, 330, 331]. Mechanical components, including strain, electronic contractility, and piezoelectric biosensors, are commonly employed to quantify contractile force and beating dynamics [157, 284, 297]. Electrophysiological properties are typically measured using MEA and nanocolumn arrays, which enable both intra- and extracellular recordings of field potentials and pacing responses with precision [157, 185, 297].

Biochemical parameters can be measured using in-line, on-line, or off-line sensors. In-line sensors, directly integrated into the MPS, and on-line sensors, which automatically analyse collected samples from the system, enable real-time data collection about intra-construct environmental and physiological parameters – oxygen, pH, fluid perfusion, electrophysiology, metabolite concentration and cardiac biomarkers secretion. These are generally fused with label-free methods, like optical microscopy, infrared spectroscopy, and surface plasmon resonance imaging (SPRi), for the assessment of tissue morphology, function and quality [24, 322]. On the other hand, off-line sensors do not allow real-time analysis and require manual sample collection and analysis using traditional tools like staining techniques, enzyme-linked immunosorbent assay (ELISA), flow cytometry, etc. [25, 322, 332, 333].

For vascular MPS, similar functional and biochemical parameters apply. However, additional vascular-specific readouts are necessary to ensure physiological relevance. These include measurements of vascular permeability using transendothelial electrical resistance (TEER), growth factor secretion via microlaser sensors, nitric oxide levels at the vascular lumen via nanowire arrays-based microelectrodes, and more specifically, arterial pressure profiles via on-a-chip capillary-assisted pressure sensors to assess such feature on microfluidic devices [185, 332, 334].

The integration of multimodal sensing technologies is now emerging as a powerful strategy to increase the functional complexity and biological fidelity of cardiovascular MPS. For instance, the dual-photomultiplier tube (PMT)

Table 4 Electromechanical stimulation of cardiovascular MPS: crucial design aspect

Type of MPS	Type of stimulation	Parameters	Platform to control and measure the parameters	Effect on the model	References
Cardiac MPS	Electrical	Pulse frequency Voltage amplitude Substrate conductivity	MEA Conductive scaffolds Bioreactors	Improvements on CM maturation & Electrophysiological contractile functions	[25, 289, 304–307]
	Mechanical	Passive: matrix stiffness & passive stretch Active: cyclic stretching & shear stress	Elastomeric devices with flexible anchors Bioreactors Electromechanical platform Microfluidic device	CM organization and maturation & Promotion of matrix-microenvironment interactions	[25, 289, 304–307, 312]
	Electromechanical	Integration of electrical and mechanical parameters	Stretchable and flexible MEAs	Advance CM maturation and developmental pathways stimulation	[25, 289, 304–307, 313–316]
Vascular MPS	Mechanical Stimulation	Shear stress ECM stiffness	Elastomeric devices	Induction of morphogenesis and sprouting Tight junctions' formation	[45, 317–321]

system, which has been implemented in vascularized cardiac organoids and heart-on-a-chip platforms to enable simultaneous measurement of oxygen consumption, extracellular field potentials, and contractility [127, 335, 336]. Other innovative technologies have also advanced real-time, multi-parameter monitoring in cardiac models. For example, the 3D arrhythmia-on-a-chip model—described in the HoC section—combines force sensing with advanced optical mapping of action potential conduction and contractile dynamics, capturing key parameters such as conduction velocity, beat frequency, and mechanical outputs [188]. Such platforms including the required functional and biochemical measurements enable high-fidelity correlation of metabolic, electrical, and mechanical functions of cardiovascular MPS underscoring novel mechanistic pathways into (patho)physiological responses and facilitating precise drug testing.

11 Current Limitations of MPS

MPS have emerged as a bridge between traditional 2D cell culture and animal models, offering physiologically relevant data to enhance the predictive value of preclinical studies [26, 51, 337]. In CVD research, MPS hold great potential for advancing the understanding of developmental biology and pathophysiology, as well as for drug discovery, screening and toxicology [1, 39, 41]. However, their broader development and translational implementation face several key challenges, including cell sourcing, tissue maturation, vascularization, immune and nervous system integration, real-time monitoring, and scalability [1, 24, 26, 39]. Addressing these limitations is essential to fully unlocking the potential of MPS in cardiovascular research and clinical translation (Table 5).

From a biological perspective, selecting the appropriate cell source remains a major hurdle. While human primary cells offer physiological relevance, their limited availability and functional decline at higher passages restrict their application [39, 45]. On the other hand, iPSCs provide a renewable and patient-specific alternative, but face challenges related to differentiation consistency, functional maturity, and scalability [19, 45]. Advanced differentiation protocols guided through single-cell transcriptomics analysis are being developed, aiming to improve cellular maturity and functional relevance [24, 159, 338]. The incorporation of supportive mesodermal or ectodermal lineage cells into cardiovascular tissue models [338], the combination of multiple organ region–specific organoids or spheroids ("assembloids") [339], and the combination of spheroids/organoids with microfluidics ("spheroid/organoid-on-a-chip") [103, 142, 143, 338], are other strategies also being explored to

Table 5 Current limitations of cardiovascular MPS and suggested solutions to address drawbacks

	Limitations/challenges	Corresponding solutions/strategies	References
MPS general parameters	Difficulty in determining the appropriate cell source - Human Primary Cells: limited in supply and lose functionality at higher passages - hiPSCs: differentiation inconsistency, phenotypical and functional immaturity	hiPSCs present more advantages than primary cells: Development of differentiation protocols based on signalling networks and molecular markers	[1, 2, 19, 24–26, 36, 39, 41, 45, 51, 74, 159, 322, 338, 343, 345, 346, 348, 350, 351, 354, 355, 358]
	Physicochemical alterations within the system over time - Difficulty in the maintenance of long-term cultures - Variability and non-reliable data	Real-time monitoring Implementation of computational modelling (i.e. in silico approaches) Integration of multi-omics data & machine learning	
	Technical issues - Platform fabrication - Material variability - Sensor incompatibility with specific systems	Implement quality control throughout device development stages Development of high-performance materials with all the required mechanical, rheological and biological properties Development of microsensors or of platforms with integrated sensor arrays	
	Lack of standardization in protocols: - Hinder MPS reproducibility and wider adoption in academic and industrial settings	Interdisciplinary collaboration and technological development Animal cell-based MPS Clinical-trial-on-a-chip Automation and miniaturization of MPS platforms Validation and regulatory legislations	
Cardiovascular MPS specific parameters	Misrepresent the complexity of the heart and vessels native microenvironment - CM immaturity (cardiac models) - Absence of vascular network throughout the myocardium - Non-representation of immune and nervous system components	Generation of complex multicellular models able to replicate intercellular dynamics: - Assembloids - Embedding simpler models in microfluidic devices/ bioprinted constructs - Multi-organ tissue chips (systemic circulation) Generation of vascularization-like phenotypes: - Co-culture of ECs or vascular MPS within cardiac models	[1, 24, 25, 32, 36, 41, 45, 74, 83–86, 154, 159, 265, 268, 339, 340]
	Fail to recreate a fully functional vascular network - Limited nutrient and oxygen delivery - Restricted growth and function of the model - Deficient replication of mechanical forces	Develop protocols that integrate developmental biology and tissue engineering concepts: - Considerations on EC source, ECM and shear control - Incorporation of angiogenic factors (i.e. ETV2) - In vivo grafting techniques	

create more physiological and anatomically accurate models for studying CVDs and testing therapeutic interventions [24, 159].

A major challenge in cardiovascular MPS is the development of functional vascular network. The lack of fully mature, organotypic vasculature, limits efficient nutrient and oxygen efficient delivery leading to necrotic core formation, particularly in spheroids and organoid cultures. In addition, current models fail to replicate key mechanical stimuli, like wall shear stress, which are critical factors in vascular diseases like atherosclerosis [1, 32, 36, 45, 74, 159, 340]. Current approaches to generate vascularization, involve co-culture of ECs or vascular MPS within cardiac models, as well as incorporation of angiogenic factors, like ETV2 [24, 25, 154, 265, 268]. While these strategies have achieved partial success, they do not fully replicate functional vasculature. Emerging bioengineering techniques including dynamic shear control and specific endothelial and ECM interactions, show promise in enhancing vascularization and physiological relevance [24, 36, 100, 159, 268].

Additionally, the absence of immune and nervous systems represents another drawback in the fabrication of pathophysiological relevant cardiovascular MPS [1, 25, 154, 159]. The incorporation of immune cells has been largely overlooked due to their heterogeneity in morphology, function, and physiological properties [271], while neuronal cells remain underrepresented despite their involvement in cardiovascular regulation and complications [341, 342]. Recent studies have already incorporated immune cells in the culture of cardiovascular MPS, recapitulating certain immunological features of inflammatory diseases. However, these modelsare far from replacing the in vivo systems in terms of immunologic complexity [1, 25, 154, 269, 270, 273]. To accurately study the impact of the immune system in CVDs, it is necessary to integrate multi-organs in one unique system. Multi-organ tissue chips are emerging as promising platforms capable of modelling chronic inflammatory diseases, like atherosclerosis, one of the most challenging targets for drug development [32, 41, 45].

As MPS models become increasingly complex, real-time monitoring and data acquisition present additional challenges [19, 25, 343]. Long-term cultures face physicochemical alterations within the system over time, which can introduce variability and compromise data reliability [24, 45, 74]. Real-time monitoring is critical for controlling these variables, yet current platforms rely heavily on microscopy to control cell behaviour, which is limited by the deeper z-plane depth in 3D cardiovascular models [1, 24, 159]. More recently, other molecular analysis techniques, such as RNA sequencing, have been used to extract more relevant information and monitor the sample, nevertheless these methods have demonstrated to be cell destructive. As an alternative, label-free, non-destructive techniques like optical metabolic imaging (OMI), infrared spectroscopy, and surface plasmon resonance imaging (SPRi) are being explored [24, 25, 159]. Additionally, integrating microfabricated sensors into cardiovascular microfluidic devices has shown potential for real-time monitoring, though further miniaturization and increased sensibility is required to ensure compatibility with the organ chips [19, 45, 159].

Continuous interdisciplinary efforts combining stem cell biology, tissue engineering, microfluidics, and systems biology have been essential to enhance the potential of MPS as a transformative tool for cardiovascular research and drug development. Addressing significant biological and technical challenges not only increase the physiological relevance of cardiovascular MPS but also accelerate their transition from experimental platforms to robust, predictive models in preclinical and clinical settings.

12 Translational Bottlenecks and Regulation

Despite encouraging progress, the widespread adoption of cardiovascular MPS in both academic and industrial settings remains limited, primarily due to lack of standardization. The increasing complexity of these systems together with variability in design methods, materials, culture conditions and readout techniques pose a significant challenge for cross-studies comparisons [19, 36, 39, 74]. The specific peculiarities of each system are often difficult to replicate, resulting in inconsistencies and reduced reproducibility across laboratories. These limitations compromise confidence in MPS as reliable tools for translational research [344].

Automation and miniaturization of MPS platforms could enhance scalability and throughput, thereby enhancing reproducibility and enabling broader adoption. However, these technological improvements all still in early stages of development and implementation [2, 19, 345, 346]. Thus, to ensure translational potential across the existing plethora of MPS, each platform must be rigorously validated for functional performance, pharmacological relevance, and reproducibility. Without such validation, MPS cannot be reliably implemented in cardiovascular preclinical applications—such as drug screening or therapeutic development—where disease complexity demands robust and predictive models [141, 347].

To validate these human-based 3D in vitro platforms, the best reference should originate from human-derived data, including clinical, epidemiological, imaging, genomic, proteomic, or gene expression analysis [348]. When such

data are unavailable or insufficient, validation may instead rely on relevant findings derived from animal or more simplistic human in vitro models [160, 349]. Importantly, the field urgently requires clear, standardized criteria to define when MPS-derived results are acceptable for regulatory or translational use. Establishing a well-defined context-of-use (CoU) for each system, together with the development of robust qualification frameworks that demonstrate fitness-for-purpose, are critical for both approval by regulatory entities and adoption by end-users. One promising strategy is to create collections of well-characterized reference compounds aligned with specific CoUs, offering a practical means to harmonize validation across platforms [348].

As a complement to these validation strategies, animal cell-based MPS are emerging as intermediaries to cross-validate findings from human-based models before progressing to preclinical and clinical trials. Notably, they can significantly reduce animal use, as cells from a single animal can generate numerous chips, thus maximizing the impact of each animal and reducing variability between datasets [51, 350, 351]. This concept is in alignment with the 3Rs framework, a global initiative to uphold the humane treatment of animals in scientific research, based on three principals: *Replace* living animals; *Reduce* the number of animals used, when there is no available alternative, and *Refine* the experimental approaches to minimize the incidence or severity of inhumane procedures [352]. In this context, MPS are extremely valuable regarding the *Replacement* principle by offering a viable non-animal alternative, considerably bypassing the need to consider the *Reducing* or *Refining* aspects of this framework [353].

These novel concepts are being increasingly incorporated into the regulatory framework. Remarkably, the FDA (USA) passed the Modernization Act 2.0, stating that mandatory animal experiments are no longer required in drug development before proceeding into clinical trials [354]. This resolution is aligned with 3Rs principle and emphasizes that the use of alternative platforms may be complementary to the more traditional and established animal models. Globally, regulatory authorities in regions such as the European Union, Canada, Brazil, South Korea, and Japan have also made legislative strides toward the use of alternative models. Both the FDA and EMA now allow submissions based on MPS-generated data, reinforcing their integration into the development of new pharmaceutical products [348].

Taken these together, the translational potential of MPS is still limited by challenges in standardization and reproducibility. Nevertheless, through coordinated efforts and the establishment of robust validation and regulatory strategies, these barriers can be progressively overcome, paving the way for MPS broader impact in biomedical research and clinical translation.

13 Future Perspectives

Looking ahead, computational modelling in the context of MPS is emerging as a powerful tool to enhance data interpretation, increase system predictability, and ultimately strengthen their translational relevance. The integration of in silico approaches shows promise to model heterogeneous diseases and to predict effective drugs, ultimately preventing or reversing CVD progression [25, 41, 355]. Computational hiPSC-CM models have demonstrated potential in improving the understanding of electrophysiology mechanisms, intracellular ion dynamics, and early-phase cardiotoxicity detection [25, 356, 357]. Future integration of spatial multi-omics data (*e.g.,* genomics, proteomics, metabolomics, transcriptomics) with machine learning and artificial intelligence could further refine predictive modelling and accelerate drug discovery [25, 159, 358]. Additionally, "clinical-trial-on-a-chip" platforms—patient-specific tissue chips derived from iPSCs—are emerging as valuable tools for optimizing clinical trial design, particularly for rare diseases [19, 41, 359]. By enabling personalized drug testing across different clinical trial phases, these individualized chips could revolutionize precision medicine.

Ultimately, the future success of MPS for cardiovascular research and clinical applications depends on overcoming the biological, technological and regulatory limitations. Ongoing efforts continue to highlight their transformative potential for human disease modelling, drug development and personalized medicine, bridging the gap between experimental research and clinical application [1, 24, 25, 159]. Future advancements will enable development of cardiovascular models that precisely replicate the biological, mechanical, and electrical activity of cardiac tissue, integrating vascular networks within complex tissue architecture.

14 Conclusion

In this review, we have reported various approaches for generating 3D multicellular systems, representing a rapidly evolving field. We have highlighted the increasing diversity of MPS types and their respective applications in cardiovascular research, and key considerations for developing physiologically relevant models. The continuous expansion of this field underscores the importance of interdisciplinary collaborations between developers, end users, and

regulatory bodies. Such cooperation is crucial for unlocking the full potential of MPS in biomedical research and particularly in preclinical studies and therapeutic development.

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Declarations

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