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3D bioprinting in iPSC-derived cardiac models: Advances, challenges, and future directions

Running title: Next-generation cardiac models from patient-specific iPSCs

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Abstract

Heart disease is a major global health challenge and is one of the leading causes of morbidity and mortality worldwide. Significant research efforts have focused on elucidating its underlying mechanisms, developing novel therapeutic approaches, and advancing drug discovery methods. The emergence of induced pluripotent stem cells (iPSCs) has provided powerful tools for modeling cardiovascular diseases, enabling mechanistic studies, drug screening, and personalized treatment strategies. More recently, three-dimensional (3D) cardiac models, including organoids, engineered heart tissues, microfluidic chip platforms, and bioprinted constructs, have further advanced cardiac tissue modeling by offering physiologically relevant platforms for drug testing and disease modeling. This review highlights recent advances in diverse iPSC-derived 3D cardiac models for disease modeling, drug discovery, and high-throughput screening, with particular emphasis on 3D bioprinting technologies that enable the spatially controlled fabrication of multicellular, biomimetic cardiac tissues with tunable structural and functional properties. Specifically, we focus on key aspects of cardiac bioprinting, including printing modalities, the rheological and mechanical properties of bioinks, achievable printing resolution and cell density, strategies for vascularization, and post-printing tissue maturation. We also discuss the integration of cutting-edge technologies, including 3D cardiac models and artificial intelligence (AI)-driven analyses, to improve the predictive accuracy of these models. The development of iPSC-derived cardiac models, combined with emerging bioengineering and computational technologies, provides a platform for cardiovascular research. In particular, computational algorithms, including machine-learning and artificial-intelligence models, are increasingly being used in the assessment of drug-toxicity and cardiotoxicity, complementing these patient-specific experimental systems. Their applications in drug screening, cardiotoxicity assessment, and precision medicine may help accelerate therapeutic development and improve patient outcomes.

Keywords: Induced pluripotent stem cells; Bioprinting; Engineered heart tissue; Heart disease modeling; Drug discovery

1. Introduction

Heart disease continues to be a significant global health challenge, consistently ranking among the leading causes of morbidity and mortality worldwide. Despite advances in diagnostics, pharmacotherapy, and interventional strategies, the heterogeneity of cardiac disorders continues to limit the effectiveness of standard treatment paradigms. Inherited cardiomyopathies, for instance, display diverse genetic and phenotypic presentations, often resulting in unpredictable clinical outcomes and variable drug responses.¹⁻³ These limitations highlight the urgent need for more predictive, patient-tailored platforms to accelerate the development of precision medicine in cardiology.^{4,5} To address these issues, researchers have undertaken innovative efforts to understand the complexities of cardiac pathologies, develop new therapeutic interventions, and transform drug discovery processes.^{4,6} One significant advancement in this field is the use of patient-specific induced pluripotent stem cells (iPSCs) to create personalized cardiomyocytes.^{7,8} This enables the development of highly individualized cardiac models, providing unprecedented opportunities to investigate the genetic basis of various cardiac disorders.^{9,10} This paves the way for tailored treatment strategies and precision medicine approaches.^{4,11} Recent progress in three-dimensional (3D) culture systems has further advanced the field.¹²⁻¹⁴ Traditional two-dimensional cardiomyocyte cultures, while informative, lack the structural, electrophysiological, and biomechanical complexity of the human myocardium.^{6,15} In contrast, 3D cardiac models, including organoids, engineered tissues, bioprinted constructs, and organ-on-a-chip platforms, can replicate key features of myocardial architecture, as well as the interactions between different cell types and the physiological microenvironment.^{12,16,17} These systems support the study of dynamic processes such as electrical conduction, contractility, and mechano-transduction under conditions that closely approximate *in vivo* physiology, thereby enhancing the accuracy of drug testing and helping to elucidate disease mechanisms.^{5,13,17-19} Together, these features make 3D iPSC-based platforms uniquely suited to advancing precision medicine in cardiology.

Beyond biological innovation, the integration of computational tools is transforming the field of cardiac research.⁴ Artificial intelligence (AI) is being used increasingly to analyze large-scale datasets generated from iPSC-derived models, including transcriptomics and proteomics. AI-driven approaches recognize patterns and predict drug-disease interactions, thereby accelerating drug repurposing and identifying novel therapeutic candidates, as well as supporting the simulation of patient-specific cardiac physiology. These advances highlight the collaboration between biological and computational sciences in advancing predictive and

personalized medicine.^{20,21} In this review, we highlight recent breakthroughs and ongoing challenges in the development of patient-specific iPSC-derived cardiac models. We focus on their applications in inherited cardiomyopathies, advances in 3D tissue platforms such as organoids, tissue engineering, bioprinting and organ-on-a-chip systems, and the emerging role of AI in drug discovery and predictive modeling. By synthesizing current knowledge across these domains, we aim to provide a comprehensive perspective on how the integration of stem cell biology, 3D tissue platforms, and computational technologies is advancing precision medicine in the field of heart disease modeling with patient-specific iPSCs.

2. Heart disease modeling using patient-specific iPSCs

Inherited cardiomyopathies, such as hypertrophic, dilated, arrhythmogenic, left ventricular noncompaction, and restrictive cardiomyopathy, represent a leading cause of sudden cardiac death and heart failure in young adults.^{2,3,22,23} Their heterogeneous genetic background and variable clinical presentation pose significant diagnostic and therapeutic challenges. Traditional models such as animal systems often fail to fully recapitulate human-specific pathophysiology, limiting their translational relevance.⁴ Recent progress in iPSC technology has enabled the generation of patient-specific cardiac models, opening new opportunities for studying disease mechanisms, testing therapeutics, and advancing personalized care. Here, we review the inherited cardiomyopathies and address advancements in applying cardiomyocytes derived from patient-specific iPSCs, offering insights into the genetic basis of cardiac pathologies. We focus on identifying genetic mutations across various cardiac disorders using patient-derived iPSC models, characterizing their molecular and phenotypic features through functional assays. We also explore their applications in drug screening and personalized medicine, while acknowledging current limitations of in vitro cardiomyopathy modeling and outlining strategies to refine these platforms for research and therapy development. An overview of the genetic landscape across the five major cardiomyopathies (Hypertrophic cardiomyopathy, Dilated cardiomyopathy, Arrhythmogenic cardiomyopathy, Left ventricle noncompaction cardiomyopathy, and Restrictive cardiomyopathy) is presented in Figure 1, with their clinical prevalence, causative genes, experimental approaches, and corresponding 3D modeling strategies summarized in Table 1.^{9,11,24}

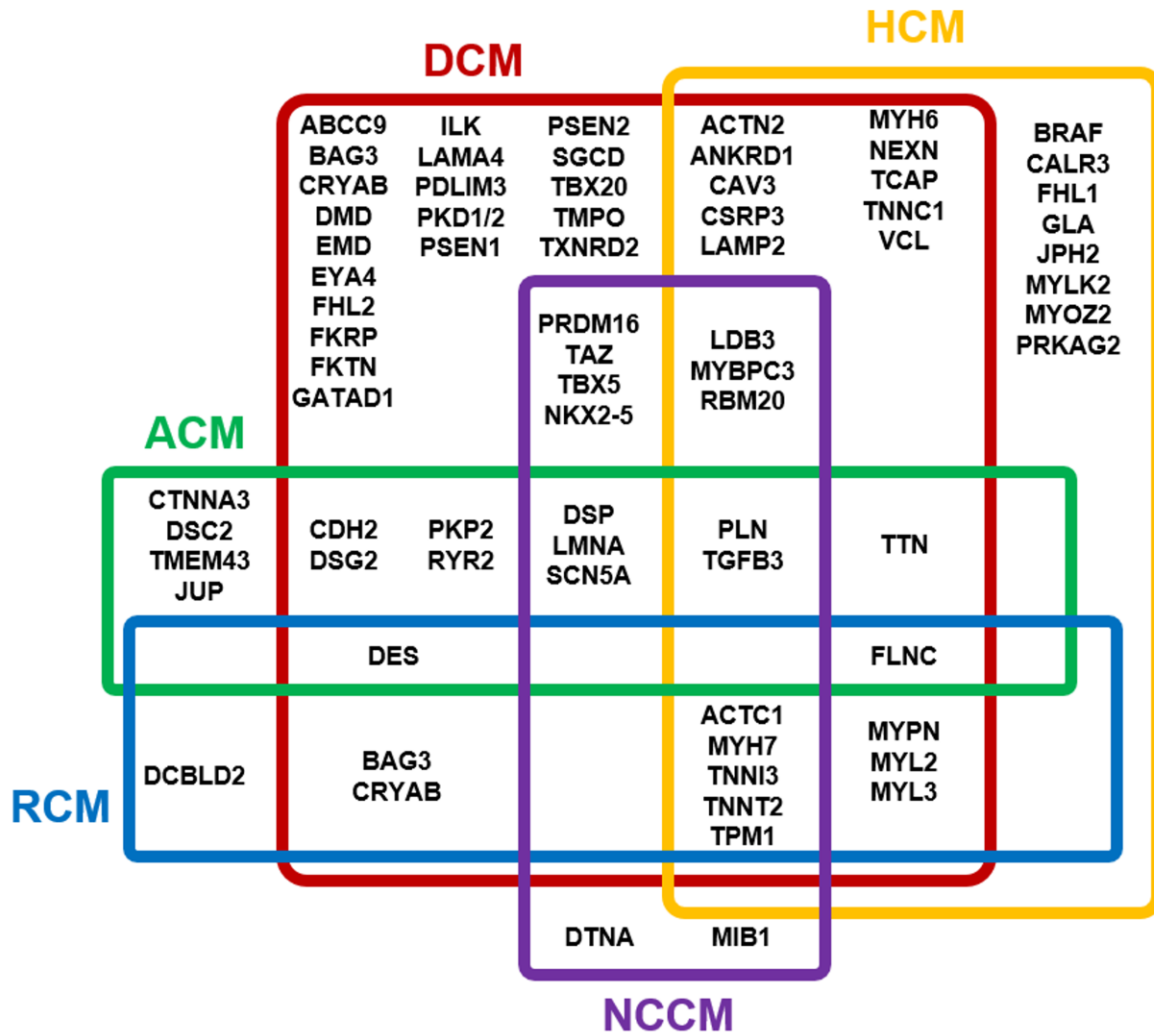


Figure 1. Genetic landscape across the five major inherited cardiomyopathies. Genes associated with HCM, DCM, ACM, RCM, and NCCM(LVNC) are shown, with overlapping regions indicating genes implicated in more than one disease. Adapted with permission from Van Tintelen et al.²⁵ with updated information from recent studies. Abbreviations: HCM, hypertrophic cardiomyopathy; DCM, dilated cardiomyopathy; ACM/ARVC, arrhythmogenic (right ventricular) cardiomyopathy; LVNC/NCCM, left ventricular non-compaction cardiomyopathy; RCM, restrictive cardiomyopathy.

Table 1. Genetic cardiomyopathies modeled with iPSC-derived cardiomyocytes: 3D modeling platforms and functional assessment approaches.

Heart Disease Types					
Cardiomyopathy		Affected	3D Modeling	Experimental approach	Ref
Hypertrophic Cardiomyopathy	HCM	1 per 500	EHT	Contractile force · sarcomere shortening, [Ca ²⁺] transients, arrhythmia/AP	26-29
Dilated cardiomyopathy	DCM	1 per 250	EHT/ Microtissue/ Oragnoid	Contractility · force, sarcomere·mitochondria, [Ca ²⁺] transients	28,30-33
Arrhythmogenic cardiomyopathy	ACM	1 per 2,000 ~5,000	EHT/ Microtissue	Contractile force, conduction velocity, gap junction (Na ⁺ current)	34-36
Left ventricular non-compaction / Non-compaction Cardiomyopathy	LVNC /NCCM	1 per 500~5,000	EHT	Contractility, arrhythmia, [Ca ²⁺] handling	27,37,38
Restrictive cardiomyopathy	RCM	~2–5% of all cardiomyopathies	EHT /Organoid	Relaxation, motion analysis, [Ca ²⁺] oscillation	39-41

This table summarizes representative inherited cardiomyopathies modeled using patient-specific iPSC-derived cardiomyocytes. For each subtype, HCM, DCM, ACM, LVNC(NCCM), and RCM, the estimated prevalence, the 3D culture platforms employed (e.g., engineered heart tissue [cardiac strip], microtissue, and organoid), and the corresponding functional assessment approaches are listed. Functional readouts span electrophysiological measurements (patch clamp, action potential recording, multi-electrode array, conduction velocity), calcium handling ($[Ca^{2+}]$ imaging and transients), and contractile/mechanical analyses (sarcomere shortening, contractility, force measurement, and relaxation/motion analysis). Abbreviations: EHT, engineered heart tissue; MEA, multi-electrode array; AP, action potential.

2.1. Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is a common inherited disease affecting 1 in 500 individuals, characterized by ventricular wall thickening and myocardial disarray. Diagnosis relies on electrocardiogram (ECG) and echocardiography, which can reveal left ventricular hypertrophy, large QRS complexes, and T-wave inversion.^{2,42,43} Current treatments, including β -blockers and surgical options such as septal myectomy, have limited efficacy and potential adverse effects.⁴⁴ Pathogenic variants in sarcomeric genes such as MYH6, MYH7, MYBPC3, TNNT2, and TNNI3 drive phenotypic heterogeneity.^{43,45-48} For example, MYH7 mutations have been associated with increased cardiomyocyte size and disrupted sarcomere architecture, whereas MYBPC3 mutations can alter gene expression, splicing, and electrophysiological

properties.^{46,49,50}

Recent advances have leveraged patient-derived iPSCs to generate cardiomyocytes (iPSC-CMs) and 3D platforms such as cardiac organoids, engineered heart tissue, and tubular constructs.^{26,51} These systems reproduce hallmark HCM mechanisms: MYBPC3 splice variants revealed non-canonical splicing leading to transcript instability, while studies in affected siblings carrying the same MYBPC3 truncation showed that modifier variants such as MYH7 can determine distinct clinical expressivity. Furthermore, the myosin inhibitor mavacamten rescued HCM phenotypes in both 2D iPSC-CM and 3D engineered-tissue models, and it was subsequently approved by the FDA for obstructive HCM on the basis of the phase 3 EXPLORER-HCM trial.^{26,29,52,53} Importantly, this mavacamten evidence spans both two- and three-dimensional formats: normalization of hypercontractility has been demonstrated in three-dimensional engineered heart tissues as well as in two-dimensional single-cell and monolayer preparations. This distinction is translationally relevant, because tissue-level loading and mechanics differ substantially between the two formats. As a concrete example of how computational parameterization can localize the mechanistic defect of a mutation, patient-derived engineered heart tissues expressing the thin-filament variant TPM1 E192K—interrogated together with molecular-dynamics simulations and multiscale cross-bridge modelling—revealed a loss of cross-bridge inhibition that produced cellular hypertrophy, hypercontractility, and diastolic dysfunction, all of which were reversed by chronic mavacamten treatment.⁵⁴ Mechanistically, mavacamten stabilizes the energy-conserving super-relaxed (SRX) state of myosin, whose destabilization by MYH7 variants underlies the hypercontractile, energetically stressed HCM phenotype,⁵⁵ and mavacamten correspondingly preserved length-dependent activation and improved diastolic function in human engineered heart tissue.⁵⁴

Functional assays such as calcium transient analysis, patch clamp electrophysiology, and sarcomere shortening measurements are widely applied to evaluate contractility, arrhythmic risk, and pharmacological responsiveness.^{51,52,56} Together, these studies establish iPSC-based models not only as a means of recapitulating HCM pathogenesis but also as promising tools for drug discovery and therapeutic optimization.^{26,44,51}

2.2. Dilated cardiomyopathy

Dilated cardiomyopathy (DCM), affecting an estimated 1 in 250 individuals and most commonly manifesting in middle age, is caused by genetic factors, drugs, pregnancy

complications, and infections. DCM leads to ventricular enlargement and inefficient contraction, diagnosed by electrocardiogram(ECG), chest X-ray, or echocardiography, revealing features such as left ventricular dilation and systolic dysfunction.^{22,57} Treatments are primarily medical, including ACE inhibitors, diuretics, and β -blockers, or surgical interventions such as pacemaker implantation and heart transplantation.^{22,57} Among genetic causes, TTN mutations are especially prevalent, while variants in TNNT2, LMNA, PLEKHM2, and SCN5A also contribute to disease pathogenesis through impaired sarcomere integrity, nuclear envelope abnormalities, autophagy defects, or ion channel dysfunction.^{37,58-63}

Recent advances using iPSCs have enabled patient-specific disease modeling of DCM.^{30,61,62} TNNT2-mutant iPSC-CMs reproduce calcium-handling defects, reduced contractility, and sarcomeric disorganization, which can be partially rescued by β -blockers or SERCA2a overexpression.^{30,59,60} LMNA-mutant iPSC-CMs exhibit nuclear envelope deformation, mitochondrial dysfunction, reactive oxygen species accumulation, and arrhythmias, highlighting oxidative stress as a therapeutic target.⁶¹ PLEKHM2-mutant iPSC-CMs reveal impaired autophagy, downregulation of contractile and calcium-handling genes, and defective sarcomere maturation.³⁷ Additionally, iPSC lines derived from patients with SCN5A mutations provide a platform to dissect the role of sodium channel dysfunction in DCM and arrhythmogenesis.⁶³ Collectively, these patient-derived iPSC models recapitulate key molecular and functional defects of DCM and serve as powerful tools for elucidating disease mechanisms, drug testing, and precision medicine approaches.^{30,58,61,62}

2.3. Arrhythmogenic cardiomyopathy

Arrhythmogenic cardiomyopathy (ACM) is a hereditary cardiac disease with a prevalence of 1 in 2,000–5,000 individuals, more common in males, and often presenting with arrhythmias or sudden cardiac death in young adults and athletes. Traditionally referred to as arrhythmogenic right ventricular cardiomyopathy, it is now recognized as a progressive disorder that can affect both ventricles. Pathologically, ACM is marked by myocyte loss, fibrofatty replacement, and electrical dysfunction.^{3,63,64} Genetic mutations in desmosomal proteins, most notably PKP2, DSP, DSG2, and DSC2, account for the majority of cases, while nondesmosomal genes such as FLNC, OBSCN, and SCN5A have also been implicated. Diagnosis is made through ECG, echocardiography, and cardiac MRI, which reveal conduction abnormalities, ventricular dilation, or structural remodeling. Management primarily targets arrhythmia prevention and reduction of sudden cardiac death risk.^{3,63-65}

Recent advances have increasingly leveraged human iPSC-based models to recapitulate ACM phenotypes. iPSC-derived cardiomyocytes carrying desmosomal mutations display impaired junction assembly, disrupted calcium handling, and reduced conduction velocity. Engineered cardiac tissues generated from these cells reproduce arrhythmogenic features such as contractile dysfunction and stress-induced arrhythmias, while pharmacological interventions targeting pathways such as Wnt/ β -catenin signaling have demonstrated the ability to restore cell–cell coupling and improve electrical propagation. Beyond cardiomyocytes, iPSC-derived epicardial cells and fibroblasts have been applied to model fibrofatty remodeling, a hallmark of ACM, showing enhanced adipogenic differentiation and structural remodeling. In three-dimensional contexts, microtissues and engineered heart tissues incorporating these cell types have further demonstrated arrhythmic behavior under pacing stress, faithfully reflecting patient tissue phenotypes.^{36,65,66}

Together, these patient-specific iPSC-based 2D and 3D platforms not only reproduce key ACM features, including fibrofatty remodeling, arrhythmogenesis, contractile dysfunction, and inflammation. They also provide valuable systems for testing therapeutic strategies, such as targeting Wnt/ β -catenin signaling or modulating mechano-transduction pathways.^{36,66}

2.4. Left ventricular noncompaction

Left ventricular noncompaction (LVNC), or noncompaction cardiomyopathy, is a rare congenital disease affecting an 1 in 500~5000 individuals, criterion-dependent number of people and is characterized by excessive trabeculations in the left ventricle.⁶⁷⁻⁶⁹ Genetic contributors include mutations in transcription factors (NKX2-5, TBX20), sarcomere proteins (MYH7), and ion channels, which affect cardiac development and contractile function.⁶⁸⁻⁷¹

Recent studies have applied patient-derived iPSC models to investigate LVNC pathogenesis. For example, iPSC-CMs from patients carrying PLEKHM2 mutations exhibit impaired sarcomere organization, reduced calcium transient amplitude, and abnormal autophagy flux, recapitulating contractile and maturation defects observed clinically.³⁷ Similarly, Kodo et al. demonstrated that iPSC-CMs harboring a PRDM16-Q187X nonsense variant display impaired proliferation, increased apoptosis, and disorganized sarcomeres, linked to dysregulated TGF- β signaling. These findings were further validated in CRISPR knock-in mouse models, which showed underdeveloped compact myocardium and embryonic lethality in homozygotes.⁷²

Together, iPSC-based systems have provided new mechanistic insights into LVNC by revealing how autophagy dysregulation and altered transcriptional networks contribute to defective myocardial compaction.^{37,72} Moreover, these patient-specific models offer translational platforms for exploring therapeutic targets such as modulation of autophagy and TGF- β signaling.⁷²

2.5. Restrictive cardiomyopathy (RCM)

Restrictive cardiomyopathy (RCM) is an exceptionally rare form of cardiomyopathy, affecting an estimated 2–5% of all cardiomyopathy cases. It is characterized by rigid ventricular walls leading to diastolic dysfunction with preserved systolic function. Diagnosis typically relies on ECG and echocardiography, which reveal biatrial enlargement and impaired ventricular filling. Etiologies include genetic mutations and infiltrative diseases, with variants in BAG3, DES, CRYAB, and FLNC linked to protein aggregation, myofibrillar disarray, and impaired autophagy. Current treatments focus on symptom management and slowing disease progression, while heart transplantation remains the only curative option.^{73,74}

Recent studies have employed patient-specific iPSC models to capture the pathophysiology of RCM. iPSC-derived cardiomyocytes harboring pathogenic mutations, particularly in TNNT3, reproduce hallmark diastolic defects. For example, iPSC-CMs carrying the TNNT3-R170W variant exhibit prolonged calcium reuptake, reduced relaxation kinetics, and abnormal contractile force ratios compared with isogenic controls. When these cells are integrated into engineered heart tissue (EHT), they display increased diastolic force and impaired relaxation, faithfully modeling the clinical phenotype of diastolic dysfunction. Importantly, correction of the mutation or overexpression of wild-type TNNT3 rescues relaxation defects, underscoring the potential of gene therapy strategies.⁴⁰

Collectively, iPSC-based 2D and 3D models of RCM provide the first robust in vitro systems for recapitulating impaired relaxation.^{40,73,75} These platforms not only advance mechanistic understanding of how sarcomeric protein mutations disrupt diastolic function but also serve as translational tools to evaluate therapeutic interventions, including targeted gene correction and protein replacement strategies.⁴⁰

3. 3D tissue platforms using patient-specific iPSCs

Building on the patient-specific iPSC-based models discussed above, 3D tissue platforms further extend our ability to recapitulate human cardiac physiology beyond the limitations of conventional 2D adherent cultures, which lack diverse cell types such as inflammatory cells in myocarditis.²⁹ The structural features, advantages, and disadvantages of the major 3D cardiac modeling approaches discussed throughout this section, including organoids, engineered tissue strips, patches, films, chambers, tubes, heart-on-a-chip systems, and bioprinted constructs, are summarized in Figure 2.^{4,6,76,77} Such 3D platforms can incorporate the major cardiac cell types, including cardiomyocytes (CMs), endothelial cells (ECs), and fibroblasts, enabling more faithful recapitulation of tissue morphogenesis.^{6,13,78,79} These 3D models serve as versatile platforms for cardiovascular research, supporting investigations into cardiac development, disease pathogenesis, and pharmacological responses. Furthermore, the use of patient-derived cell sources holds promise for regenerative medicine, personalized therapy, and patient-specific disease modeling.^{4,6,12,76,80}

3D Culture Systems		Applications	Advantages	Disadvantages
Various types of tissue engineering	Organoid 	Disease modeling, developmental study, viability and apoptosis readouts	Patient-specific, In vivo-like complexity and architecture, Self-organization	Variable/low reproducibility, Hard to reach maturity, Lacks vasculature
	Strip 	Contractile force measurement, optical mapping, Ca ²⁺ transients	Non-invasive force readout, Anisotropic organization, Amenable to electrical stimulation	Low throughput, Drug absorption by silicone posts, High cell demand
	Patch 	Myocardial repair and engraftment, large-area optical mapping	Covers and supports injured area, Promotes regeneration, Amenable to electrical stimulation	Host integration challenges, No direct force measurement, Limited vascular integration
	Film 	Contraction readout via film curvature, high-throughput screening	High-throughput, Flexible thin platform, Large surface for optical mapping	Limited 3D (thinness), No direct force measurement, Requires patterning
	Chamber 	Pressure-volume loops, optical mapping, Ca ²⁺ transients	Clinically-relevant readouts, Physiological loading, Amenable to electrical stimulation	Labor-intensive, Complex fabrication, Specialized equipment
	Heart on-a-chip 	Real-time functional monitoring, dynamic perfusion, drug and toxicity screening	Physiological fluidic flow, Precise microenvironment control, Real-time readouts	Lacks vasculature, Difficult to scale up, High cost and expertise, hard for HTS
	Bioprinting 	Customizable, scalable tissue fabrication, complex architecture, co-culture constructs	High precision and resolution, Customizable architecture and gradients, Various bioinks	Lacks vasculature, Cell and material challenges, Immature tissue, costly

Figure 2. Comparison of 3D cardiac tissue engineering platforms: structural features, advantages, and limitations. This table compares the major 3D platforms used to engineer cardiac tissue from iPSC-derived cardiomyocytes. Eight configurations are summarized (organoid, strip, ring, patch, film, chamber, heart-on-a-chip, and bioprinting) each illustrated by its representative structure alongside its principal advantages and limitations. Organoids and chamber models offer greater structural and developmental fidelity but face challenges in vascularization, maturation, and scalability. Strip, ring, patch, and film models provide simpler, reproducible formats well-suited to contractile measurement and high-throughput drug screening, with the ring configuration

additionally supporting self-sustained re-entrant waves that promote maturation and enable arrhythmia modeling, at the cost of full three-dimensional complexity. Heart-on-a-chip systems integrate microfluidic control for real-time, multiparametric readouts, while bioprinting enables patient-specific, spatially defined constructs but still contends with biocompatibility and vascularization hurdles. Together, these platforms span a continuum between experimental tractability and physiological relevance, and the choice of model depends on the specific biological question and intended application.

Existing 3D cardiac platforms can be broadly categorized into scaffold-based approaches, which rely on biomaterial supports to guide tissue architecture, and scaffold-free approaches, which depend on intrinsic self-organization of cells.^{12,16,17,80} Across both categories, these systems have been employed to investigate cardiac development, model inherited and acquired cardiac diseases, and evaluate pharmacological responses at the tissue level.^{12,80} Despite their growing sophistication, challenges in model fidelity, reproducibility, and scalability remain central obstacles to widespread translational use, motivating ongoing refinements in cellular composition, biomaterial design, and bioengineering strategy. The remainder of this section examines each class of 3D cardiac platform in turn, with an emphasis on iPSC-based implementations and their disease-modeling applications.

3.1. Cardiac organoid

Cardiac organoids represent a significant advancement in cardiac tissue engineering, providing three-dimensional self-organizing structures that recapitulate aspects of heart development and disease.^{12,17,80} Various approaches have been developed to generate these structures, employing different cellular sources, scaffold materials, and bioengineering strategies tailored to producing three-dimensional heart-like tissue.⁸¹ Major challenges include faithfully replicating the structural and functional complexity of native myocardium, particularly at the level of cell-type composition, chamber-like organization, and vascularization.^{80,82} Robust characterization is essential for evaluating maturation and functional fidelity, and relies on high-resolution imaging, transcriptomic and proteomic profiling, and electrophysiological assessment.^{80,82} Collectively, these organoid platforms have become central tools for developmental biology, disease modeling, and drug discovery.^{12,17,76,80,82}

3.1.1. Organoid

Heart organoids, created in laboratories using stem cells or other cell types, are simplified three-dimensional models that mimic aspects of the human heart.^{12,17,80} For example, self-assembling heart organoids derived from hiPSCs have been shown to differentiate into cardiomyocytes and endothelial cells.⁸³ Under specific conditions, these stem cells self-organize into structures resembling components of the developing heart, including chamber-like architecture.^{75,84} Such organoids have become valuable platforms for studying cardiac development, modeling diseases, and assessing drug toxicity, thereby providing deeper insights into cardiac function and pathology.^{12,75,80,84}

Despite their promise, heart organoids remain simplified systems and do not fully replicate the complexity of a mature human heart.^{12,17,80} Critical challenges include incomplete vascularization, lack of standardization, and limited scalability for translational use.^{80,82} Recent work has proposed that the size and differentiation period of hiPSC-derived cardiac organoids are decisive for their reliability in drug testing, identifying small-scale organoids differentiated for extended periods as optimal benchmarks. Other studies have also shown that integrating vascular cells into cardiac organoids improves structural maturation and disease modeling potential. Addressing these limitations is essential for enhancing the efficacy and translational relevance of cardiac organoids, particularly in regenerative medicine and precision healthcare.^{12,17,80,82}

3.1.2. Cardioid

In 2021, the Mendjan lab introduced the first chamber-like cardiac organoid, termed "cardioid," developed from human induced pluripotent stem cells.⁷⁵ These self-organizing cardioids, replicating early embryonic development of the left ventricle, mark a significant advancement in understanding human cardiogenesis and heart disease. Generated through tightly controlled modulation of WNT, ACTIVIN, BMP, and retinoic acid signaling, cardioids self-organize into chamber-like structures with a defined cavity, recapitulating key aspects of cardiac mesoderm specification and ventricular morphogenesis without the need for external scaffolds or matrix support.^{75,84}

Beyond developmental modeling, cardioids have provided new insights into cardiac injury

and regeneration; cryoinjury of cardioids triggers fibroblast activation, extracellular matrix deposition, and scar-like tissue formation that recapitulate hallmarks of human myocardial infarction *in vitro*.⁷⁵ More recent multi-chamber cardioid systems further extend this approach by co-developing atrial and ventricular compartments alongside an inflow tract and outflow tract, capturing chamber-specific gene expression programs and the structural relationships present in the early heart. Despite these advances, cardioids remain limited in size, lack mature vasculature and a functional conduction system, and do not yet incorporate immune or neural components, restricting their applications in long-term physiological modeling and large-scale drug screening. Nevertheless, by combining chamber-specific morphogenesis with patient-derived iPSCs, cardioids offer a uniquely tractable platform for studying congenital heart defects, exploring regenerative responses, and evaluating drug effects on chamber-level cardiac function.^{75,84} Recent strategies are beginning to address these limitations; for example, vascularized, chamber-like cardiac organoids assembled by combining iPSC-derived vascular spheroids with cardiomyocytes have yielded constructs that contain multiple cardiac cell types, beat spontaneously, and have been used to model cardiac injury, fibrosis, and dose-dependent doxorubicin toxicity, illustrating how integrated vascular co-development can extend organoid maturity and disease-modeling utility.⁸⁵

3.2. Tissue engineering

Engineered heart tissues (EHTs) are sophisticated *in vitro* models designed to emulate cardiac muscle tissues, playing a key role in understanding cardiac function under various stimuli.^{6,13} These models are developed through the combination of cells and biomaterials and include diverse forms such as strips, rings, patches, films, and chambers.^{6,13,78,79} Each model serves a distinct purpose in replicating and studying the complexities of cardiac function and structure, offering unique insights into cardiac physiology. Despite their advantages, these models also present limitations in fully mimicking the intricate structure and function of the human heart.^{6,13}

3.2.1. Strip model

The strip model represents one of the earliest and most widely used configurations in engineered cardiac tissue research.^{13,28} In this approach, cardiac cells are embedded into elongated, ribbon-like constructs that facilitate straightforward handling and reproducible

measurement of contractile performance. Because of their geometry, these tissues are particularly suitable for quantifying force generation, stress–strain relationships, and length–tension properties, making them a valuable system for biomechanical assays and drug testing.^{28,86}

A key advantage of this model is the ability to mount tissues between force transducers or flexible posts, enabling high-precision readouts of twitch kinetics and pharmacological responses under controlled loading conditions.^{28,87} Studies using this setup have shown that engineered strips can recapitulate length-dependent activation and even reproduce aspects of anisotropy when aligned with matrix scaffolds.^{86,87} Importantly, strips constructed from human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have provided insights into patient-specific disease phenotypes. For instance, tissues generated from titin-mutant hiPSCs demonstrated impaired contractility consistent with sarcomere insufficiency, while other patient-derived strips have been used to reveal altered responses to β -adrenergic stimulation. These findings highlight the ability of strip models not only to reproduce fundamental aspects of muscle mechanics but also to capture disease-relevant mechanisms and drug sensitivities. Laser-cut decellularized myocardium has also been adapted into strip-like forms that preserve native fiber orientation, enabling systematic investigation of mechanotransduction and functional anisotropy in both healthy and diseased contexts. Despite their limitations, strip models remain indispensable for probing the fundamentals of cardiac muscle physiology. Their reproducibility, accessibility, and compatibility with high-throughput measurements make them an important bridge between single-cell studies and more sophisticated three-dimensional platforms.^{28,30,87}

Nevertheless, the strip model has notable limitations. Its essentially two-dimensional architecture does not fully capture the complex three-dimensional arrangement of cells, extracellular matrix, and vascular networks found in the native heart.¹³ This constrains its utility for studying tissue–tissue interactions, chamber formation, and long-term maturation.^{13,78} In addition, oxygen and nutrient diffusion remain less physiologically relevant compared with thicker constructs or organoid systems. While culture conditions, scaffold innovations, and electrical or mechanical conditioning can partially improve maturity, strip models generally fall short in replicating the structural and functional complexity of intact myocardium.^{13,78}

3.2.2. Ring model

The ring model adopts a looped, ring-shaped engineered tissue structure, which more closely simulates the natural alignment of cardiac muscle fibers. This configuration is particularly advantageous for studying muscle contraction dynamics and the effects of mechanical loading and unloading on cardiac tissue. In addition to these applications, ring-shaped constructs generated from hiPSC-CMs have been shown to self-assemble into stable myocardial rings that exhibit synchronized contractions, robust cTnT expression, and long-term electrophysiological activity. These constructs provide a controlled environment to evaluate how engineered tissues respond to continuous mechanical tension along the circumferential axis, thereby offering insights into the relationship between geometry and functional output. A notable feature of the ring model is its ability to support the emergence of self-sustained traveling waves of electrical activity. These circulating waves act as an intrinsic pacing mechanism, continuously stimulating cardiomyocytes and promoting their structural and functional maturation without the need for external pacing systems. Experimental studies have demonstrated that such traveling waves lead to elongated sarcomeres, improved calcium handling, and upregulation of cardiac-specific proteins, highlighting the potential of ring-shaped tissues as platforms to enhance hiPSC-CM maturation. Beyond maturation, the persistence and stability of these waves resemble reentrant excitation patterns observed in arrhythmogenic hearts, making the ring model a useful tool for investigating the mechanisms of rhythm disorders and for testing anti-arrhythmic interventions.^{88,89}

Despite these advantages, the ring model remains a simplified representation of the heart. Its architecture does not capture the full three-dimensional complexity of myocardial chambers or the heterogeneous cell populations present in vivo.^{88,89} Moreover, traveling waves in long-term cultures can become unstable, leading to irregular beating patterns that limit reproducibility.⁸⁹ These constraints restrict its application to targeted questions of contraction dynamics, electrophysiology, and arrhythmia modeling rather than holistic simulation of cardiac function. Nevertheless, its simplicity, reproducibility, and compatibility with hiPSC-based approaches position the ring model as a powerful intermediate platform, bridging the gap between basic cell culture systems and more advanced three-dimensional cardiac constructs.^{88,89}

3.2.3. Patch model

The patch model comprises flat, sheet-like engineered tissues that are frequently designed to mimic the myocardial wall or to repair infarcted regions. Owing to its planar and broad architecture, the patch can be directly applied to the epicardium, positioning it as a promising tool for regenerative therapies. Beyond serving as passive reinforcement, recent strategies have emphasized its role as an active biological scaffold. Constructs seeded with pluripotent stem cell–derived cardiomyocytes, vascular progenitors, and stromal cells have demonstrated organized electromechanical coupling, anisotropic alignment, and robust contractile force generation. For example, micropatterned fibrin scaffolds loaded with hiPSC-cardiomyocytes promote connexin-43 expression, improved calcium handling, and synchronized contractions. Likewise, 3D-printed auxetic patches seeded with iPSC-derived cardiomyocytes have shown superior mechanical compliance with the dynamic beating of the heart, enhanced vascularization in the infarct border zone, and significant attenuation of adverse ventricular remodeling in vivo. Together, these examples highlight how design innovations and cell-based strategies can transform the patch model into both a therapeutic and investigative platform.^{90,91}

Despite these advances, critical challenges remain unresolved. Seamless electromechanical integration with host myocardium is difficult to achieve, and incomplete coupling may increase the risk of conduction block or arrhythmia. Immune reactions to biomaterials or transplanted cells, insufficient long-term engraftment, and poor vascularization within thick constructs continue to hinder translation to clinical practice. Furthermore, although auxetic and micropatterned scaffolds provide improved mechanics and alignment, scaling patches to human-relevant dimensions while maintaining cell viability and function remains a substantial obstacle.^{90,91} Among the most promising engineering solutions are electrically conductive scaffolds: gold nanowires incorporated into cardiac patches improve cell-to-cell electrical communication and synchronous contraction,⁹² and graphene-oxide/gold-nanosheet composites roughly double scaffold conductivity and, after implantation into infarcted myocardium, improve conduction velocity and contractility while upregulating the gap-junction protein connexin-43,⁹³ directly targeting the gap-junctional coupling deficits that underlie poor electromechanical integration.

Overall, the patch model stands out as one of the most clinically promising approaches in cardiac tissue engineering. By combining pluripotent stem cell–derived cardiomyocytes with

advanced biomaterial designs and integrative cues for maturation, engineered patches hold significant potential for myocardial repair following infarction. Yet, achieving durable host integration, minimizing arrhythmogenic risks, and ensuring stable regenerative benefit will be essential milestones before these constructs can move from experimental models to routine clinical use.^{90,91}

3.2.4. Film model

The film model represents a thinner, film-like structure in engineered cardiac tissues, primarily designed to study the laminar organization of cardiac cells.⁹⁴ Its planar format facilitates high-resolution observation of cell–cell interactions, contractility, and the influence of microenvironmental cues, making it well-suited for mechanistic studies and pharmacological screening.^{86,94} Recent innovations have advanced the film model beyond simple monolayers by introducing muscular thin film (MTF) platforms, where aligned cardiomyocytes are cultured on flexible substrates that bend in response to contractile forces. These systems enable precise quantification of tissue stress, conduction velocity, and pacing responses in a scalable format.⁹⁴ Micromolded gelatin hydrogels, for example, support long-term culture of human iPSC-derived cardiomyocytes, extending functional activity for weeks while preserving sarcomere organization and metabolic reserve capacity. Other approaches have incorporated nanofabricated topographies or elastomeric scaffolds to guide anisotropy, enhance connexin-43 expression, and improve calcium handling, thereby capturing more physiologically relevant behavior in thin-film constructs. These advances highlight the utility of the film model not only for acute pharmacological testing but also for chronic cardiotoxicity studies requiring extended culture duration.^{94,95}

Despite its strengths, the film model remains limited by its reduced dimensionality. Unlike thicker constructs or organoid systems, films cannot fully replicate the three-dimensional heterogeneity, vascularization, or mechanical loading experienced in native myocardium. This restricts their applicability to organ-level modeling or regenerative applications. Nevertheless, because of their reproducibility, compatibility with high-throughput drug screening, and ability to reveal fundamental cell–cell and cell–matrix dynamics, film-based models remain an indispensable platform within cardiac tissue engineering, complementing more complex 3D systems.^{13,94}

3.2.5. Chamber model

The chamber model represents one of the most advanced approaches in cardiac tissue engineering, aiming to replicate the structural and dynamic features of heart chambers, including their hollow geometry, wall curvature, and blood flow dynamics.^{96,97} By capturing pressure–volume relationships and fluid mechanics, this model provides a physiologically relevant platform for investigating cardiac anatomy and function.⁹⁶⁻⁹⁸ Its construction, however, requires highly sophisticated methodologies such as molding, scaffold design, and integration of cell-laden hydrogels, making it among the most resource-intensive models in the field.⁹⁶⁻⁹⁹

Engineered heart tissues (EHTs) incorporated into chamber-like systems have shown significant promise in both fundamental and translational applications. Patient-derived hiPSCs have been used to model cardiomyopathies such as Barth syndrome, Duchenne muscular dystrophy, hypertrophic cardiomyopathy, and short QT syndrome, as well as metabolic and acquired cardiac disorders.⁹⁷⁻⁹⁹ These constructs not only reproduce disease-specific phenotypes but also enable non-invasive, real-time monitoring of contractile force, conduction, and pharmacological responses, positioning them as valuable tools for drug discovery and high-throughput screening. Moreover, chambered constructs have been advanced toward pump-like function, with chambered organoids and human chambered muscle pumps (hChaMPs) exhibiting macroscale beating, pressure–volume loop generation, and drug responsiveness. Such progress brings the chamber model closer to applications in regenerative medicine, experimental cardiac assist devices, and therapeutic testing, especially when combined with immune-compatible iPSC sources.^{96,97,99-101}

Despite these advances, challenges such as ensuring sufficient wall thickness, robust vascularization, and stable electromechanical coupling with host myocardium remain significant hurdles. Even so, the chamber model underscores the frontier of engineered cardiac tissues, where structural fidelity and physiological function intersect with translational potential.^{96,97}

3.3. Organ-on-a-chip

The heart organ-on-a-chip (OoC) represents a microfluidic platform that integrates cell biology, biomaterials, and engineering to emulate the structural and functional complexity of

the human heart.^{102,103} By guiding cells to assemble into three-dimensional, physiologically relevant tissues, these systems enable multiparametric readouts, including contractility, electrophysiology, and calcium handling under controlled microenvironmental conditions.¹⁰²⁻
¹⁰⁴ Building on advances in tissue engineering, the engineering techniques described above are now being incorporated into chip-based platforms, simplifying complex experiments and making them more accessible for broad experimental use. Compared with traditional cell cultures or animal models, OoCs provide a more accurate in vitro representation of human cardiac physiology, though challenges remain in material selection, device miniaturization, and balancing fabrication complexity with biological fidelity.

Applications of cardiac OoCs have been rapidly expanding in drug development and disease modeling. Single-organ platforms are valuable for assessing targeted responses to pharmacological interventions, while multi-organ configurations connect cardiac tissue with liver, vasculature, or kidney modules to capture systemic drug distribution and toxicity.^{102,105} Integration of hiPSC-derived cardiomyocytes has further enabled the modeling of patient-specific diseases, drug sensitivities, and arrhythmia phenotypes. For example, advanced systems now incorporate electrical pacing and real-time biosensors to improve cardiomyocyte maturation and allow continuous, non-invasive monitoring of tissue performance.^{102,103,105}

Recent studies have pushed the boundaries by incorporating immune components and vascularized structures.¹⁰² For instance, immune-integrated heart-on-a-chip models have successfully recapitulated SARS-CoV-2-induced myocarditis, identifying mitochondrial DNA release as a driver of dysfunction and demonstrating the therapeutic efficacy of endothelial exosomes.¹⁰⁶ Such examples illustrate how OoCs can move beyond drug screening toward modeling complex, multi-cellular disease environments.^{102,106} Beyond viral myocarditis, heart-on-a-chip systems have been extended to other complex injury processes; for example, an epicardium–myocardium heart-on-a-chip has been used to model ischemia–reperfusion injury, showing that an intact epicardial layer reduces cell death, preserves function during simulated reperfusion, and contributes migratory, myofibroblast-like cells to the injured tissue.¹⁰⁷

Despite these advances, replicating the full biological authenticity of the human heart remains difficult, with ongoing challenges in long-term stability, vascularization, and scalability. Nevertheless, the convergence of stem cell technology, biosensors, and microfluidic engineering positions the cardiac OoC as a pivotal tool for translational research.

As fabrication methods diversify, these platforms are increasingly being applied as experimental testbeds, enabling complex cardiac and systemic studies that were once impractical with conventional in vitro or in vivo approaches.¹⁰²⁻¹⁰⁴

3.4. Bioprinting

Bioprinting in the context of heart tissue creation is a sophisticated process that precisely layers cells, biomaterials, and bioactive molecules to reconstruct tissue architectures resembling the heart's structural complexity.¹⁰⁸ Its strength lies in customization, particularly the ability to fabricate patient-specific cardiac tissues that can advance disease modeling and drug screening. Unlike conventional tissue formats such as organoids, strips, patches, films, and chambers, bioprinting offers higher spatial precision and structural control, enabling the fabrication of complex architectures, including perfusable vascular conduits and microengineered cardiac constructs.^{108,109} Representative bioprinting strategies for cardiac applications are illustrated in Figure 3, including patch-, chamber-, vascular-, and multi-layer/film-based designs for cardiac patches, chamber-like constructs, tissue-engineered blood vessels, and heart-on-a-chip platforms.¹⁹

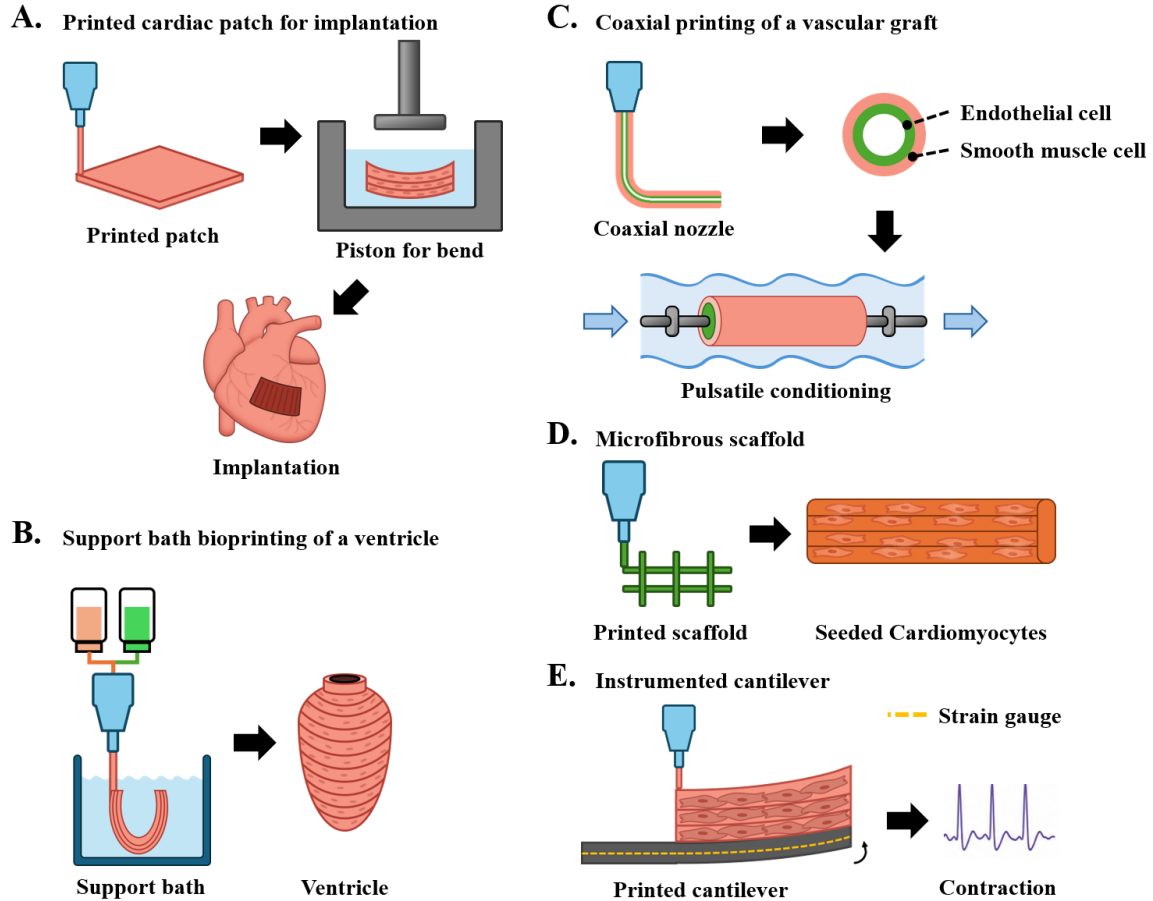


Figure 3. Representative bioprinting strategies for cardiac tissue engineering. (A) Cardiac patch model. A 3D-bioprinted patch is shaped into a curved, physiologically adaptable (4D) geometry that conforms to the epicardial surface and is implanted in vivo in a myocardial infarction model.¹¹⁰ (B) Ventricle model. hiPSC-cardiomyocytes embedded in a collagen–hyaluronic acid bioink are printed within a thermo-responsive support bath (embedded/in-gel bioprinting) to generate a functional, contractile ventricle-shaped construct.¹¹¹ (C) Vascular model. Coaxial bioprinting positions ECs in the luminal layer and VSMCs in the outer layer, forming a perfusable TEBV that matures under pulsatile conditioning.¹¹² (D) Microfibrous scaffold. A bioprinted microfibrous scaffold is seeded with cardiomyocytes to engineer aligned, endothelialized myocardial tissue for heart-on-a-chip applications.¹¹³ (E) Instrumented cantilever. A cantilever fabricated by multimaterial 3D printing integrates an embedded strain gauge that continuously records the contractile activity of the printed cardiac tissue.¹⁰⁹ Abbreviations: hiPSC, human induced pluripotent stem cell; EC, endothelial cell; VSMC, vascular smooth muscle cell; TEBV, tissue-engineered blood vessel. Schematic created by the authors.

Among the most widely studied applications are iPSC-derived cardiac patches, which have shown proper electrical coupling and functional contraction after transplantation into injured myocardium, positioning them as promising tools for regenerative therapy.^{90,91} Bioprinted chamber-like constructs have further advanced physiological modeling by replicating pressure–volume relationships and chamber morphologies more accurately than traditional

mold-based tissues.^{96,108} Vascular bioprinting techniques have also enabled the generation of perfusable, lumenized cardiac tissues, where iPSC-derived cardiomyocytes are patterned around printed vascular channels to sustain nutrient exchange and support long-term function. Importantly, recent approaches integrating iPSC-derived spheroids into self-healing hydrogels have demonstrated high-cell-density cardiac microtissues capable of mimicking post-infarction fibrosis and testing pro-regenerative therapies such as microRNA delivery.^{90,91,108}

Finally, bioprinting has been increasingly combined with organ-on-a-chip technology, where bioprinted iPSC-cardiac strips or patches embedded into microfluidic systems allow quantitative, high-throughput analyses of contractility, electrophysiological activity, and pharmacological responses. This integration underscores the translational relevance of bioprinted models, establishing them as pivotal platforms not only for cardiac disease research but also for drug discovery and regenerative medicine.^{102,108,109}

3.4.1. Printing modalities

Cardiac bioprinting has been implemented through several distinct modalities that differ in achievable resolution, cell density, and throughput. Extrusion-based printing remains the most widely applied approach. Using freeform reversible embedding of suspended hydrogels (FRESH), collagen has been printed at a filament resolution of approximately 20 μm to reconstruct components of the human heart, including perfusable vasculature and tri-leaflet valves¹¹⁴. Extrusion of personalized decellularized extracellular matrix (dECM) bioinks derived from a patient's own omentum has enabled thick (~ 2 mm) vascularized cardiac patches and small-scale cellularized hearts, with sacrificial gelatin used to template perfusable lumens of ~ 300 μm ¹¹⁵. To reconcile print fidelity with physiological cell density, embedded and sacrificial-writing strategies such as sacrificial writing into functional tissue (SWIFT) organize iPSC-derived organ building blocks at densities approaching 200 million cells/mL while writing perfusable branching channels of 400 μm to 1 mm, yielding constructs up to 4 mm thick¹¹⁶. Light-based approaches, including stereolithography and digital light processing, cure cell-laden resins to pattern fine microarchitecture; a beam-scanning stereolithographic strategy produced a 4D self-morphing cardiac patch from a 5% GelMA/15% PEGDA resin whose modulus lies within the range of native myocardium¹¹⁰. Laser-assisted bioprinting (laser-induced forward transfer, LIFT) enables nozzle-free, high-precision cell positioning; LIFT-patterned endothelial and mesenchymal cells on a polyester-urethane-urea patch improved vascularization and cardiac function after myocardial infarction *in vivo*¹¹⁷. Each modality thus offers a characteristic balance of resolution, cell density, construct thickness, and throughput,

summarized in Table 2. In parallel, advances in functional, stimuli-responsive polymers are expanding the materials toolbox for structurally sophisticated cardiac constructs; for example, polyamide-12 meta-composites combine excellent mechanical performance with shape-memory behavior, flame retardancy, and 3D/4D printability, illustrating how emerging structural polymers can complement the cell-laden bioinks of Table 2 in fabricating patient-specific architectures.¹¹⁸

Table 2. Comparison of 3D bioprinting modalities for cardiac tissue. Entries summarize representative primary studies for each modality; several references are listed where multiple studies illustrate the same approach.

3D Bioprinting Modalities for Cardiac Tissue					
Modality	Working principle	Material	Resolution	Key features	Ref
Extrusion-based (FRESH, dECM)	Continuous filament deposition; support-bath embedding (FRESH) enables printing of soft, low-modulus inks	Collagen, alginate/fibrin, decellularized ECM (dECM), sacrificial gelatin	20 μm filament (FRESH); 200~300 μm sacrificial lumens	Full-organ anatomy · perfusable vasculature & valves · patient-specific dECM patch (implantable)	114,1
					15,11
					9,120
Embedded / sacrificial writing (SWIFT)	Writing sacrificial or cell-dense ink within a matrix; removal leaves perfusable networks	Gelatin sacrificial ink; iPSC organ building blocks; carbohydrate glass	Channels 400 μm ~1 mm	Near-physiological cell density · perfusable branching channels · cm-scale, on-chip perfusion	116,1
					21,12
					2
Light-based (SLA / DLP / volumetric)	Photopolymerization of cell-laden resin; layer-wise (SLA/DLP) or tomographic volumetric curing	GelMA and PEG-based photoresins (e.g., GelMA/PEGDA)	μm -scale features / voxels	Fine microarchitecture · 4D self-morphing patch · multivascular networks · seconds-scale build	110,1
					23,12
					4
Laser-assisted (LIFT)	Nozzle-free laser-induced forward transfer; droplet-wise, high-precision cell placement	Cell suspensions on donor ribbon; polyester-urethane-urea patch	Single-cell ($\sim\mu\text{m}$) positioning	High-precision patterning · high post-transfer viability · improved vascularization & function after MI (in vivo)	117,1
					25
Multimaterial / organ-on-chip	Multimaterial extrusion of matrix, cells and soft sensors within perfusable microdevices	Piezoresistive/conductive inks, GelMA microfibers, ECM	100~500 μm features	Integrated sensing · myocardium · perfusion bioreactor · drug/toxicity screening (chip)	108,1
					09

3.4.2. Bioink rheology and formulation

A central determinant of print fidelity is the rheological behavior of the bioink, which must be shear-thinning enough to flow through the nozzle yet recover sufficient yield stress to retain the printed geometry. Equally important, the crosslinked construct should approximate the mechanical stiffness of native myocardium, whose passive elastic modulus is on the order of

10 to 15 kPa, whereas fibrotic or scar-like tissue is considerably stiffer at roughly 35 to 70 kPa¹²⁶. Gelatin methacryloyl (GelMA) is widely used because its stiffness is readily tuned across this physiological window, with reported elastic moduli of approximately 2.5, 15.3, and 30.1 kPa at 5%, 10%, and 15% (w/v), respectively, supporting cardiomyocyte alignment and maturation. Tissue-specific dECM bioinks better recapitulate the biochemical composition of myocardium; a cardiac dECM bioink could be tuned across a compressive modulus of 13.4 to 89 kPa spanning healthy-to-fibrotic states while maintaining greater than 94% viability of encapsulated iPSC-derived cardiomyocytes after 7 days¹²⁷, and heart-derived dECM bioinks promote early cardiomyocyte differentiation and support pre-vascularized cardiac patches^{128,129}. Matching bioink rheology and post-crosslinking stiffness to these targets is therefore essential for both printability and downstream tissue function.

3.4.3. Quantitative comparison of platforms

Despite rapid progress, quantitative benchmarking across bioprinting modalities remains limited, because primary studies report heterogeneous and often non-overlapping metrics. Table 2 compiles the values directly reported in representative primary studies, including the working principle, material/bioink composition, printing resolution, and the distinctive functional feature of each modality (e.g., vascularization, perfusion, and organ-on-chip integration). This comparison highlights a consistent trade-off: extrusion-based FRESH achieves the finest filament resolution (~20 μm) but is typically applied at moderate cell densities, whereas embedded-writing SWIFT achieves near-physiological cell densities (~200 million cells/mL) and centimeter-scale perfusable constructs at coarser channel dimensions (400 μm to 1 mm). The scarcity of standardized, cross-comparable measurements, particularly for post-printing viability expressed as a percentage and for functional maturation, is itself an important gap that the field must close to enable rigorous platform selection.

3.4.4. Perfusion and vascularization

A defining advantage of bioprinting over self-organizing or mold-based formats is the ability to build vasculature directly into thick constructs, mitigating the diffusion limit of roughly 100 to 200 μm that otherwise produces necrotic cores. Sacrificial and embedded strategies template hollow, endothelializable channels: SWIFT constructs contain perfusable branching channels (400 μm to 1 mm) that can be actively perfused at 100 to 500 $\mu\text{l/min}$, sustaining viability

throughout 4mm thick tissue that would otherwise develop a necrotic core within 12 h without perfusion¹¹⁶. Personalized dECM printing similarly generated perfusable ~300 μm lumens within cardiac patches¹¹⁵, and FRESH-printed collagen supported multiscale vasculature from capillary to organ scale¹¹⁴. Endothelial lining of these channels is frequently incomplete, however, and long-term maintenance of an anti-thrombotic, barrier-competent endothelium within bioprinted cardiac tissue remains an active challenge.

3.4.5. Post-print maturation

Bioprinting does not by itself confer maturity; printed iPSC-cardiomyocytes generally begin in an immature state and require sustained conditioning to approach adult-like structure and function. Reference adult human cardiomyocytes exhibit a relaxed sarcomere length of approximately 2.2 μm , a resting membrane potential near -90 mV, and a twitch force on the order of 44 mN/mm¹³⁰, targets that engineered and printed tissues only partially attain. Intensity-training regimens applied to engineered tissue have driven early-stage iPSC-cardiomyocytes to a physiological sarcomere length of 2.2 μm , an adult-like mitochondrial content (~30% area), and a conduction velocity of approximately 25cm/s within four weeks, although absolute force remained below the adult benchmark.⁸⁷ For directly bioprinted tissue, centimeter-scale ring- and ventricle-shaped constructs printed from a collagen-hyaluronic-acid bioink developed well-organized sarcomeres and sustained spontaneous contraction over several months¹¹¹, while the post-printing electromechanical and metabolic conditioning summarized in Section 4 remains necessary to advance maturation further.

4. Assessment and analysis of 3D models using patient-specific iPSCs

Recent advances in 3D culture systems have further enhanced the potential of iPSC-derived models.^{4,6-8} Unlike 2D monolayers, 3D constructs such as organoids, engineered tissues, bioprinted structures and organ-on-a-chip platforms capture the key features of tissue architecture and cell-to-cell interactions, as well as dynamic mechanical and biochemical cues.^{6,13,17} These models can mimic electrophysiological synchronization, contractile mechanics and tissue remodeling, making them particularly valuable for studying complex pathophysiological processes in the cardiovascular system. Furthermore, 3D platforms offer enhanced predictive capabilities for drug screening and toxicity testing as they more closely

resemble *in vivo* conditions and patient-specific responses. For example, patient-specific iPSC-derived cardiac organoids and engineered heart tissues have already been evaluated for their ability to reproduce hypertrophic cardiomyopathy phenotypes, arrhythmogenic conduction defects, and drug-induced cardiotoxicity, demonstrating their translational relevance in precision medicine.^{4,6,18}

The assessment and analysis of these 3D patient-specific iPSC-derived models is critical to their successful implementation in precision medicine research.^{4,6,18} Here, we explore methods for analyzing electrophysiology, defined by Ca^{2+} ion activities, and contractility, associated with cellular motion, to provide a comprehensive understanding of heart diseases using iPSC technology.^{4,9} Electrophysiological properties and contractility are key to understanding the development of cardiomyocytes, which undergo transformations including the organization of contractile proteins, regulation of ion channels and gap junctions, and distribution of mitochondria and the sarcoplasmic reticulum. Notably, iPSC-derived cardiomyocytes from patients with long QT syndrome or Duchenne muscular dystrophy have been assessed using 3D constructs, confirming disease-specific prolongation of action potentials, irregular calcium handling, and impaired contractility that align with clinical manifestations. This is crucial both for understanding congenital heart anomalies and for generating functionally mature cardiomyocytes suitable for cell therapy and electrophysiological investigation. Cardiac contraction and relaxation depend on excitation–contraction coupling, involving calcium cycling between the cytoplasm, sarcoplasmic reticulum, and troponin. iPSC-CMs exhibit calcium transients similar to those of native cardiomyocytes, indicating intact excitation–contraction coupling. However, iPSC-CMs often display immature calcium handling, potentially due to underdeveloped sarcoplasmic reticulum and T-tubules.^{56,131}

For analyzing electrophysiology and contractility, both intracellular and extracellular electrical recordings from cardiomyocytes are employed, using techniques such as patch clamp, multielectrode array recording, fluorescence imaging, and impedance-based assays.^{56,132,133} The patch clamp technique, vital for examining ionic currents, can be applied in voltage- or current-clamp mode and tailored to specific objectives, ranging from whole-cell recordings to the study of individual ion channels.^{132,134} This approach has been applied to iPSC-derived cardiomyocytes carrying mutations in ion channel genes such as *SCN5A*, where patch-clamp recordings revealed altered sodium current density and arrhythmogenic susceptibility, validating the clinical phenotype at the cellular level.

Multielectrode arrays (MEAs) provide non-invasive, simultaneous recording of extracellular field potentials from multiple sites across a cardiomyocyte monolayer or engineered tissue, capturing depolarization, repolarization, and conduction dynamics in real time.¹³³ Compared with patch clamp, MEAs offer label-free, long-term monitoring of beating cardiomyocyte networks and high parallelization, making them well suited for cardiac safety screening and arrhythmia detection, though they have lower spatial resolution and cannot resolve subcellular ionic currents.^{133,135} In iPSC-derived engineered heart tissues, MEAs have been employed to map conduction velocity and arrhythmic events, confirming that patient-specific lines recapitulate clinically observed conduction slowing and susceptibility to re-entry circuits.^{133,135,136}

Calcium handling in cardiomyocytes is essential for heart function, with abnormalities linked to cardiovascular diseases. Computational models are increasingly used alongside experimental studies to understand cardiac calcium handling and its role in diseases, particularly cardiac arrhythmias.¹³¹ These models, developed based on experimental data, are becoming more complex, representing subcellular structures responsible for calcium handling and the effects of calcium-dependent signaling pathways.^{131,137} In addition, fluorescent calcium imaging of iPSC-derived cardiomyocytes from catecholaminergic polymorphic ventricular tachycardia (CPVT) patients has confirmed abnormal Ca^{2+} sparks and delayed afterdepolarizations, directly linking patient genetics to arrhythmia mechanisms.^{131,137,138}

5. 3D engineered cardiac models as a new platform for drug development

The process of discovering and developing new therapeutics has long relied on animal models and immortalized cell lines. Although animal models are valuable for basic mechanistic insights, they are time-consuming, expensive, and biologically distinct from humans in crucial ways.^{4,5} A significant proportion of pharmaceuticals that demonstrate efficacy and safety in rodent models ultimately fail when administered to humans, often due to unforeseen toxicity or insufficient efficacy. This translational disconnection has been identified as a major contributor to the high attrition rate in drug development, underscoring the necessity for more predictive, human-relevant systems.

The US Food and Drug Administration (FDA) has formalized this paradigm shift through a sequence of legislative and regulatory actions. The FDA Modernization Act 2.0, signed into law on December 29, 2022, removed the long-standing statutory requirement that animal testing be conducted as part of Investigational New Drug (IND) applications, formally authorizing alternatives such as cell-based assays using human iPSCs, organoids, organs-on-chips, and AI-based computational models.^{4,5,139-142} Building on this foundation, the FDA issued an official roadmap in April 2025 to phase out animal testing. This framework prioritizes AI-driven toxicity models and organoid-based safety assessments as key New Approach Methodologies (NAMs). Subsequently, the FDA Modernization Act 3.0, currently pending before Congress, would require the FDA to publish an interim final rule replacing references to "animal" tests, data, studies, models, and research with "nonclinical" tests across IND regulations.¹³⁹⁻¹⁴⁴ Together, these reforms remove the statutory barrier to using patient-specific iPSC-derived 3D platforms and AI-driven computational tools in preclinical assessment, although formal regulatory qualification of these methods remains to be established. For example, Burridge et al.¹⁴⁵ demonstrated that patient-derived iPSC-cardiomyocytes could predict individual susceptibility to doxorubicin-induced cardiotoxicity, highlighting the translational value of patient-specific models for drug safety testing.¹⁴⁵⁻¹⁴⁷

Among the alternatives currently gaining traction, hiPSC-CMs have emerged as a particularly promising avenue for cardiovascular research and preclinical testing.^{4,9,11} hiPSC-CMs offer the potential to model disease, explore the impact of genetic variation, and evaluate compound responses across diverse patient backgrounds.¹⁴⁶⁻¹⁴⁸ These cells reproduce a wide range of disease-relevant features, from the consequences of single-gene mutations to metabolic dysfunctions and altered electrophysiology, while expressing the ion channels and signaling pathways that define cardiomyocyte biology, thereby recapitulating numerous aspects of native cardiac function. Their widespread adoption in preclinical research reflects their ability to reveal meaningful, human-specific responses that are often invisible in animal systems.¹⁴⁹ Notably, engineered cardiac tissues from HCM patient iPSCs carrying MYH7 mutations have shown that treatment with the myosin inhibitor mavacamten normalizes hypercontractility, mirroring clinical outcomes and directly supporting precision therapy development.^{26,146,148,150}

3D engineered platforms using patient-specific iPSCs hold considerable potential for the development of more predictive, efficient, and humane approaches to evaluating new drugs.^{4,5,18} Despite remaining challenges, particularly with respect to maturation,

reproducibility, and scalability, the progress already achieved indicates a fundamental transformation in preclinical research. When incorporated into formats such as spheroids, strips, or chamber-like constructs, iPSC-derived cardiomyocytes demonstrate improved maturation and functional properties including synchronized contraction, electrophysiological activity, and force generation.^{28,87} Strategies for promoting maturation include prolonged culture, mechanical and electrical stimulation, metabolic conditioning, and co-culture with supportive cell types such as fibroblasts and endothelial cells. Advances in biomaterials and scaffold design are also contributing to more physiologically relevant environments.^{86,87,95} In addition, multi-organ platforms linking cardiac tissue with liver or kidney models have demonstrated how metabolic processing in one organ can modulate cardiac drug responses, suggesting more holistic applications in pharmacokinetic and toxicity assessment. Supported by the regulatory reforms advanced under the FDA Modernization Act 2.0, the FDA's roadmap to phase out animal testing, and the anticipated FDA Modernization Act 3.0, these platforms are likely to play an increasingly central role in drug development, ultimately improving translational outcomes and ensuring that new therapies are safer and more effective for patients. A representative example is the Comprehensive in vitro Proarrhythmia Assay (CiPA), which incorporated hiPSC-cardiomyocytes to better predict proarrhythmic risk and has since influenced international safety guidelines, demonstrating how patient-derived cardiac models are already reshaping regulatory decision-making.^{141,142,144} Furthermore, the integration of computational tools and AI is enhancing predictive accuracy by uncovering patterns across large datasets, thereby linking experimental results with clinical outcomes in ways that were previously unattainable.^{18,146-148,150,151} In the specific context of NAMs qualification, the 3D platforms, including organoids, engineered tissues, organ-on-a-chip, and bioprinted constructs, address several attributes emphasized by regulators: human genetic relevance conferred by patient-derived iPSCs, mechanistically interpretable functional readouts, and compatibility with standardized, higher-throughput safety assays. The principal remaining requirements for formal qualification are demonstrating reproducibility across laboratories and defining context-of-use-specific performance criteria. Scaling these platforms from research tools to industrialized assays poses distinct challenges. Three-dimensional constructs remain comparatively costly and labor-intensive to produce at scale; organoid and cardioid protocols still lack cross-laboratory standardization, contributing to batch-to-batch variability; and integrating complex three-dimensional readouts into automated high-throughput screening workflows requires further advances in assay miniaturization, imaging, and data analysis.

Addressing cost, protocol standardization, and high-throughput-screening compatibility will be essential for broad adoption of these systems in industrial drug development.

6. Artificial intelligence-driven drug discovery

AI is now widely applied in drug discovery to help identify, optimize, and validate therapeutic candidates.^{21,140} Traditional drug development pipelines are notoriously costly and time-intensive, with the majority of compounds failing in late-stage clinical trials due to unforeseen toxicity or insufficient efficacy in human populations.²¹ AI addresses these bottlenecks by integrating and extracting patterns from vast, heterogeneous datasets, including genomics, transcriptomics, proteomics, chemical libraries, and imaging data, at a scale and speed that is difficult to achieve with conventional approaches.^{140,152} The principal AI paradigms employed in drug discovery include supervised learning for property prediction, unsupervised learning for chemical space exploration, reinforcement learning for molecular optimization, and generative modeling for de novo compound design. Widely used open platforms underpinning these workflows include RDKit and DeepChem (cheminformatics and deep learning for molecular property prediction), PyTorch Geometric and DGL-LifeSci (graph neural network frameworks for molecular graphs), and the Therapeutics Data Commons (TDC; tdcommons.ai) benchmark suite, which provides standardized ADMET datasets and leaderboards for model evaluation.^{140,152} The Connectivity Map (CMap; clue.io), DGIdb (dgidb.org), and Open Targets (opentargets.org) further support AI-driven drug repurposing by enabling systematic compound–gene–disease interaction queries. The regulatory legitimacy of these approaches has been progressively codified. The FDA Modernization Act 2.0, signed into law in 2022, removed the statutory requirement for animal testing in investigational new drug applications and authorized non-animal alternatives, including AI-based computational models.^{141,142} Building on this, the FDA's April 2025 roadmap to phase out animal testing and the FDA Modernization Act 3.0 (S.355; assed by the Senate in December 2025 and the House in July 2026, though not yet enacted as of mid 2026) would further advance AI-driven NAMs as increasingly relevant to preclinical safety assessment, reflecting an ongoing shift in regulatory science.^{139,142,144} In cardiovascular medicine, integration of these AI platforms with patient-specific iPSC-derived cardiac data enables both the generation of disease-relevant biological readouts and the computational extraction of actionable therapeutic insights.^{20,21} These AI

approaches, together with their iPSC-CM inputs, prediction targets, and translational outputs, are summarized in Table 3.

Table 3. Machine-learning and artificial-intelligence approaches applied to iPSC-derived cardiomyocyte modeling and cardiac drug screening.

AI / Machine-Learning Applications in iPSC-Cardiomyocyte Models & Drug Screening					
AI/ML approach (Method)	Representative tools & models (algorithms/tools)	iPSC-CM input data	Prediction target / application	Experimental validation & clinical output	References
Supervised learning (QSAR, classification)	DNN, GNN (Attentive FP, ChemBERTa); CardioToxNet, hERG-Att	Molecular descriptors; iPSC-CM patch-clamp and MEA data	Binding affinity, hERG liability, proarrhythmia risk	Cardiac safety classification of compounds	152-156
Generative de novo design	REINVENT 4, DiffSBDD; Insilico Medicine platform	Target structure; iPSC-CM contractility readouts	Novel sarcomere modulators (MYH7/MYBPC3), RBM20 correctors	Candidate compounds advanced to experimental testing	157,158
Structure-based virtual screening	AlphaFold2, DiffDock, GNINA, FEP+	Predicted structures of cardiac targets (RBM20, LMNA, ion channels)	Binding poses, hit prioritization	Phenotype-validated hits in 2D/3D iPSC-CM models	156,159
ADMET & cardiotoxicity prediction	ADMETlab, DeepPK, DeepHIT; CiPA (CSS, O'Hara-Rudy)	SMILES; iPSC-CM action-potential and Ca ²⁺ -transient data	QT prolongation, Torsade-de-Pointes / arrhythmia risk	Regulatory cardiac safety assessment (CiPA framework)	160-164
Multi-omics & knowledge integration	Seurat, Scanpy, MOFA+, SPOKE, BioBERT	scRNA-seq, ATAC-seq, proteomics from iPSC-CM	Disease-specific regulatory networks, druggable nodes	Mechanistic hypotheses for iPSC-based validation	165-167
Phenotypic imaging & digital twins	CellProfiler, CellPose (CNN); LSTM/Transformer on MEA; CHASTE, OpenCOR	High-content iPSC-CM images; MEA time-series	Contractility/sarcomere and arrhythmia classification; in silico drug response	Automated profiling; digital-twin drug-response prediction validated against iPSC-CM data	168-172

For each approach, representative tools/models, iPSC-CM input data, prediction targets, and experimental/clinical outputs are listed, with supporting references in the rightmost column. For a broader discussion of iPSC-based cardiac disease modeling and drug discovery, and of how computational and machine-learning methods are being integrated into these platforms, we respectfully refer to the reviews by Teles et al. and Vo et al.^{173,174}

Among supervised learning approaches, deep neural networks (DNNs) have demonstrated strong performance in quantitative structure–activity relationship (QSAR) modeling, predicting binding affinities, selectivity profiles, and off-target liabilities from molecular descriptors and fingerprints.^{21,152} Established platforms include Schrödinger's Glide/QikProp, OpenBabel, and the ChEMBL database (ebi.ac.uk/chembl), which currently provides curated bioactivity data for approximately 2.5 million compounds (ChEMBL release 35, December 2024) and is widely used to train and benchmark predictive models. Graph neural networks

(GNNs) represent molecular topology as atom–bond graphs; frameworks such as Attentive FP, MPNN (Message Passing Neural Networks), and SchNet implemented in PyTorch Geometric or DGL-LifeSci achieve state-of-the-art results on molecular property benchmarks including MoleculeNet. Transformer-based models, such as ChemBERTa and MolBERT (both available on HuggingFace), as well as Uni-Mol, treat SMILES strings as chemical language sequences, learning context-dependent pharmacological grammar with strong generalization across drug discovery tasks. In the cardiovascular domain, GNN-based models have been applied to screen compound libraries for hERG channel liability and sodium current modulation using training data from iPSC-CM patch-clamp and MEA assays; specialized tools such as CardioToxNet and hERG-Att provide purpose-built cardiac safety classifiers.^{156,175} Ensemble methods combining multiple model architectures further improve predictive reliability, particularly for sparse datasets typical of rare cardiomyopathies.¹⁵²

Generative AI models have opened a frontier in de novo drug design, enabling computational creation of novel molecules with pre-specified physicochemical and biological properties.^{152,157} Key platforms include REINVENT 4 (github.com/MolecularAI/REINVENT4), an open-source reinforcement learning framework by AstraZeneca for goal-directed molecular generation; GuacaMol and MOSES, standardized benchmarking suites for evaluating generative model diversity and novelty; and DiffSBDD and TargetDiff, diffusion-based structure-conditioned models that generate ligands directly within protein binding pockets. The Insilico Medicine platform (insilico.com), integrating generative chemistry with AI-based target identification, produced rentosertib (formerly ISM001-055), a first-in-class TNIK inhibitor and one of the first AI-discovered, AI-designed drugs to reach clinical proof-of-concept. In the GENESIS-IPF Phase 2a trial (n = 71, 22 sites), rentosertib demonstrated safety and efficacy in patients with idiopathic pulmonary fibrosis, with results published in Nature Medicine in 2025.¹⁵⁸ For cardiac applications, generative pipelines have been explored to design selective sarcomere modulators targeting MYH7 and MYBPC3 mutation landscapes characterized by iPSC-CM contractility readouts, as well as novel RBM20 splicing correctors navigated through AI-guided chemical space. Molecular optimization is further enabled by Bayesian optimization frameworks (Ax; ax.dev), multi-objective genetic algorithms in SELFIES representation, and cloud-based platforms such as Schrödinger's LiveDesign and Atomwise, which combine generative design with scalable docking and ADMET filtering.^{152,157}

Structure-based drug discovery has been transformed by AlphaFold2 (alphafold.ebi.ac.uk), introduced by DeepMind in 2021 and expanded in collaboration with EMBL-EBI through 2022 to deposit predicted structures for over 200 million proteins. This coverage has since grown to more than 214 million entries in the AlphaFold Protein Structure Database.¹⁵⁹ AlphaFold2-derived structural models, accessible via the EMBL-EBI server and integrated into Schrödinger's Maestro and OpenFold pipelines, have enabled virtual screening against previously 'undruggable' cardiac targets, including intrinsically disordered regions of RBM20, LMNA nuclear lamins, and sarcomere assembly factors, implicated in cardiomyopathy pathogenesis.^{20,159} Traditional docking tools such as AutoDock Vina, Glide (Schrödinger), and DOCK6 are now complemented by AI-powered docking platforms including DiffDock (github.com/gcorso/DiffDock), Uni-Mol Docking, GNINA, and NeuralPLexer, which achieve improved binding pose accuracy, particularly for flexible cardiac ion channel targets. Integrated virtual screening pipelines, operationalized through Schrödinger's FEP+ (free energy perturbation), AutoDock-GPU, and the open-source HTMD (high-throughput molecular dynamics; github.com/Acellera/htmd), combine protein structure, AI scoring, and free energy calculations for rigorous hit prioritization. In the iPSC-CM context, virtual screening hits prioritized by these platforms are subsequently validated using patient-specific transcriptomic signatures analyzed through Seurat or Scanpy, ensuring biological relevance before proceeding to phenotypic assays in 2D and 3D cardiac models.^{159,165} This structure-informed, phenotype-validated workflow represents a powerful convergence of computational and experimental cardiac drug discovery.^{20,21}

Early prediction of ADMET properties and cardiotoxicity risk is a critical AI application in cardiovascular drug development, as these factors are leading causes of clinical trial attrition.^{21,176} Widely adopted AI-based ADMET platforms include SwissADME (swissadme.ch), pkCSM (biosig.unimelb.edu.au/pkcsml), ADMETlab 2.0 (admetmesh.scbdd.com), and DeepPK, which predict hepatic clearance, plasma protein binding, oral bioavailability, and renal excretion via web-accessible interfaces requiring only a SMILES string input.¹⁷⁶ For cardiac safety specifically, specialized deep learning tools include CardioToxNet (drug-induced QT and proarrhythmia prediction), DeepHIT (hERG inhibition prediction), and models within the CiPA (cipaproject.org) working group framework, trained on iPSC-CM action potential and calcium transient datasets to predict drug-induced QT prolongation and arrhythmia risk.¹⁷⁵ The CiPA initiative has formalized AI-assisted iPSC-CM electrophysiological analysis as a regulatory cardiac safety framework, replacing hERG-only

screening with a mechanistically richer, human-cell-based paradigm; analytical tools including the Cardiac Safety Simulator (CSS) and in silico cardiac AP models (O'Hara-Rudy, ToR-ORd) are central components of this pipeline.^{175,177} Multi-task deep learning frameworks, such as those built on Chemprop (chemprop.readthedocs.io) or DeepChem, simultaneously predict contractile dysfunction, calcium handling abnormalities, and mitochondrial impairment from iPSC-CM imaging and electrophysiology data, increasing safety profiling throughput.^{175,176} These AI-enhanced safety platforms, integrated with automated imaging systems such as ImageXpress (Molecular Devices), Opera Phenix (PerkinElmer), and IN Cell Analyzer (Cytiva), are increasingly explored as complementary tools for preclinical cardiac safety testing. This positioning aligned directly with the FDA's emerging NAMs framework advanced by the FDA Modernization Act 2.0 and the anticipated Act 3.0.^{20,139,141,142,144}

AI-driven multi-omics integration represents a key strategy for extracting mechanistic insight from the complex molecular landscapes of inherited cardiomyopathies.^{140,165} Single-cell multi-omics data from iPSC-CM platforms, generated using 10x Genomics Chromium (scRNA-seq), ATAC-seq, and mass spectrometry proteomics, are analyzed through Seurat (satijalab.org/seurat), Scanpy (scanpy.readthedocs.io), ArchR, and MOFA+ (Multi-Omics Factor Analysis), which decompose shared and modality-specific variance to reveal disease-specific regulatory networks.¹⁶⁵ Knowledge graph-based systems, including SPOKE (spoke.ucsf.edu), Hetionet (het.io), and Neo4j-based drug repurposing graphs, organize gene–disease–drug relationships from STRING (string-db.org), DisGeNET (disgenet.org), and ChEMBL into queryable networks, enabling systematic identification of druggable nodes within cardiomyopathy pathways.^{140,166} NLP tools including PubTator (pubtator.ncbi.nlm.nih.gov), SciSpaCy, and BioBERT mine millions of PubMed abstracts for gene–phenotype associations, adverse drug reactions, and mechanistic linkages relevant to HCM, DCM, and ACM, generating testable hypotheses for iPSC-based validation.^{21,140,167,178} Federated learning frameworks, including PySyft, FLUTE, and NVIDIA FLARE, enable AI model training across distributed iPSC-CM datasets from multiple institutions without centralizing patient data, facilitating larger training cohorts for rare cardiomyopathies while maintaining data governance compliance.^{21,140,179} Together, these multi-omics and knowledge-integration AI strategies provide a systems-level view of cardiomyopathy biology that complements the mechanistic depth of patient-specific iPSC models.^{20,165}

The deepest impact of AI in cardiac drug discovery will emerge from its tight integration with

patient-specific iPSC-derived cardiac models across the entire preclinical pipeline.^{20,21} High-content imaging platforms (ImageXpress Micro Confocal [Molecular Devices], Opera Phenix [PerkinElmer], and the IN Cell Analyzer [Cytiva]) generate terabytes of iPSC-CM phenotypic data analyzed by CNN-based classifiers such as CellProfiler, CellPose, and the Phenomic AI platform, enabling automated quantification of contractility, sarcomere organization, mitochondrial health, and nuclear morphology at single-cell resolution.^{21,180} For electrophysiological analysis, LSTM and Transformer-based models applied to MEA time-series data exported from Axion BioSystems Maestro (axionbiosystems.com) and Multi Channel Systems MEA2100 (multichannelsystems.com) platforms enable automated classification of arrhythmic events, conduction slowing, and action potential biomarkers with sensitivity exceeding manual annotation.^{20,175} Digital twin frameworks parameterized by iPSC-CM data, including CHASTE (chaste.cs.ox.ac.uk), OpenCOR (opencor.ws), and the Cardiac Electrophysiology Web Lab (cardiac.ep.cs.ox.ac.uk), simulate patient-specific drug responses in silico before experimental validation, compressing the lead optimization cycle.^{177,181-183} The digital-twin paradigm has likewise been validated in other excitable tissues, for example, a myoelectric digital twin of myocytes that generates realistic, annotated electromyographic signals to train deep-learning decoders¹⁸⁴, supporting the extensibility of data-calibrated in silico replicas to three-dimensional engineered cardiac tissues. Figure 4 illustrates the major AI methodologies in cardiovascular drug discovery: transcriptome-based repurposing (CMap/clue.io, Open Targets), generative molecular design (REINVENT, DiffSBDD), structure-based virtual screening (AlphaFold2, DiffDock), ADMET/cardiotoxicity prediction (ADMETlab, CiPA/CardioToxNet), and phenotypic iPSC-CM screening (CellProfiler, ImageXpress).

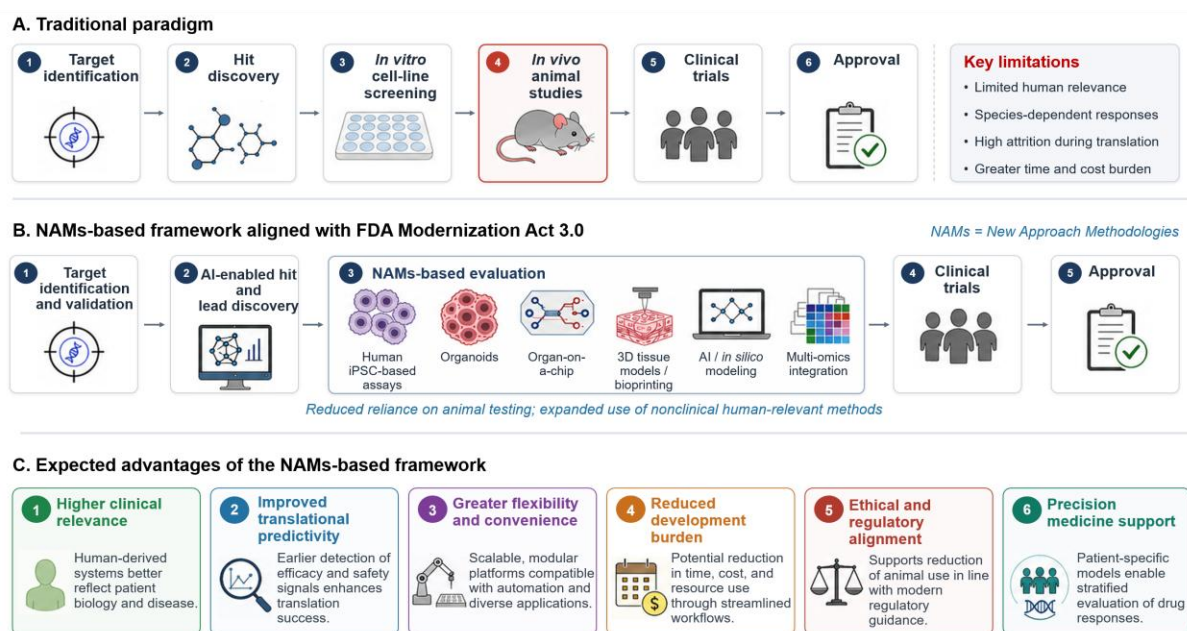


Figure 4. Transition from traditional animal-based drug development to a NAMs-based human-relevant framework in the emerging NAMs era anticipated under the FDA Modernization Act 3.0. (A) Traditional paradigm and its key limitations; (B) replacement of animal-centered nonclinical testing with human-relevant and in silico methodologies (iPSC assays, organoids, organ-on-a-chip, 3D/bioprinted tissues, AI/in silico, and multi-omics) for candidate discovery and preclinical evaluation^{139,142,183}; (C) expected advantages : clinical relevance, translational predictivity, flexibility, reduced development burden, ethical/regulatory alignment, and precision medicine support.¹⁴² Abbreviations: AI, Artificial intelligent; FDA; Food and Drug Administration, NAMs; New Approach Methodologies.

Figure 5 depicts the next-generation integrated pipeline connecting patient-derived iPSC cardiac models with multi-omics profiling, AI-driven compound identification and optimization, and iterative experimental validation, representing the convergent vision of precision cardiovascular medicine. Despite this progress, important challenges remain: model interpretability (addressed by tools such as SHAP, LIME, and GNNExplainer), limited generalizability across genetic backgrounds, regulatory frameworks still adapting to AI-generated evidence, and the need for prospective clinical validation.^{21,152} Addressing these challenges through interdisciplinary collaboration among computational scientists, iPSC biologists, cardiologists, and regulatory experts will be essential to translate AI-guided cardiovascular discoveries into clinical benefit: a translation increasingly enabled, and now formally encouraged, by the FDA's evolving NAMs regulatory framework under the Modernization Act 2.0 and 3.0.^{20,139,141,142,144} Passini et al. provided robust validation of this concept, demonstrating that *in silico* drug trials, utilizing population-level models of human ventricular action potentials, can effectively capture inter-individual variability in drug

responses. This approach successfully identified subpopulations vulnerable to repolarization or depolarization abnormalities. Notably, these simulation-based models achieved 89% accuracy in forecasting clinical arrhythmogenic risk, with the simulated biomarker shifts demonstrating strong concordance with experimental assays.¹⁶⁹

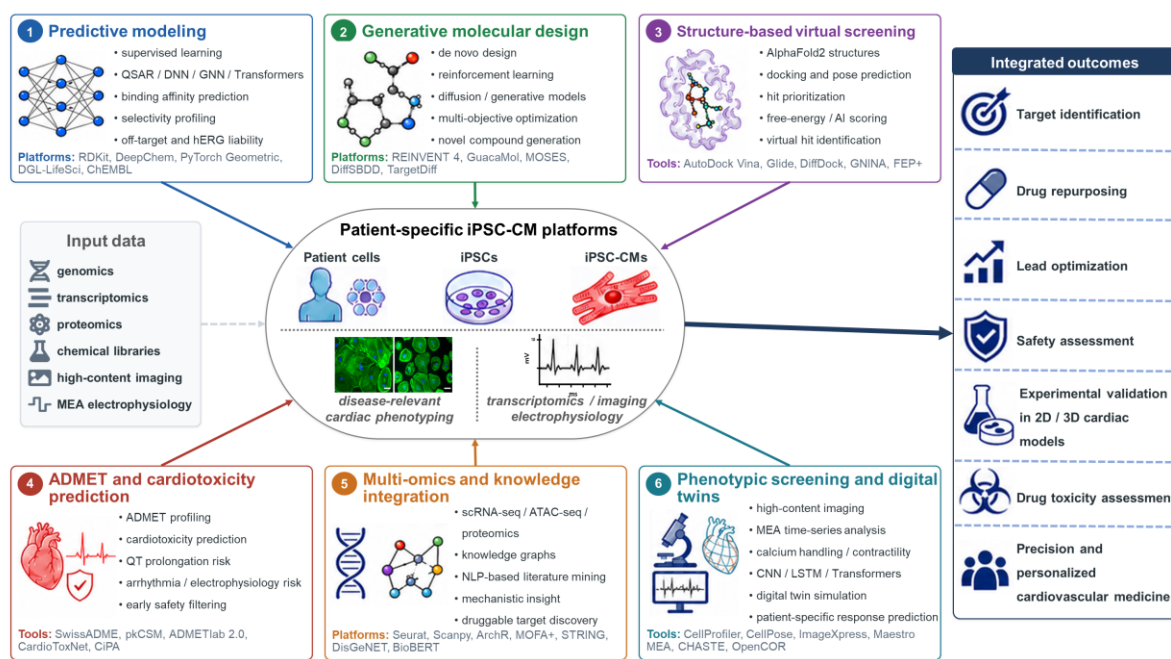


Figure 5. Artificial intelligence integrated with patient-specific iPSC-CM platforms for cardiovascular drug discovery. Six computational modules (1) predictive modelling, (2) generative molecular design, (3) structure-based virtual screening, (4) ADMET and cardiotoxicity prediction, (5) multi-omics and knowledge integration, and (6) phenotypic screening and digital twins converge on patient-derived iPSC-CM models as the central experimental hub. Input data feed the platform, which drives integrated outcomes from target identification and lead optimization to safety assessment and precision cardiovascular medicine. Representative platforms/tools appear in each module, with key challenges (interpretability, generalizability, dataset bias, regulatory adaptation, prospective clinical validation) noted below.

7. Conclusion

The integration of patient-specific iPSCs with advanced 3D cardiac tissue models has significantly advanced our ability to investigate human heart diseases in a physiologically relevant manner.^{4,7,8,10} These platforms have enabled more accurate replication of native cardiac microenvironments, offering a versatile foundation for mechanistic studies, genetic modeling, and therapeutic testing.^{4,6-8} While organoids, engineered heart tissues, and organ-on-a-chip platforms reproduce important structural and functional features of the human myocardium, 3D bioprinting offers distinct advantages through the precise spatial organization

of multiple cardiac cell types, extracellular matrix components, and vascular-like architectures.^{108,115} The role and significance of bioprinting in this field lie in its capacity to generate customizable and reproducible cardiac tissue constructs with controlled geometry, cellular composition, and biomechanical properties.^{108,114}

The transition from two-dimensional monolayer cultures to multicellular 3D systems has not only improved the predictive value of preclinical screening but also opened new avenues for precision medicine.^{4,18} In particular, bioprinted cardiac tissues can be designed to support standardized, scalable, and geometrically defined platforms compatible with drug screening and cardiotoxicity assessment.^{109,111} However, before using an integrated platform based on patient-derived iPSCs, rigorous genotype-to-phenotype attribution is crucial. To accurately attribute phenotypes to specific genotypes, researchers must consider genetic and epigenetic background variations by utilizing CRISPR-corrected isogenic controls and analyzing multiple clones across independent differentiation batches. Furthermore, factors such as sex and maturation states must be carefully standardized. We respectfully refer to the following review for a detailed discussion on how to handle these variables.¹⁸⁵⁻¹⁸⁷

AI technologies, particularly those applied to compound screening, target prioritization, and multi-omic data integration, have the potential to complement these biological models.^{20,21,140} However, their effective application depends on continuous refinement and alignment with empirical data generated from biologically complex systems such as 3D cardiac tissues.^{21,156,157} For bioprinting-based platforms, continued optimization of bioink rheological and mechanical properties, printing resolution, cell viability and density, vascularization, and post-printing maturation will be crucial for improving physiological fidelity and translational utility.^{108,129}

In conclusion, 3D tissue models hold the potential to serve as a valuable component of cardiovascular research and therapeutic development. Their convergence with high-throughput analytical tools, integrated biosensors, and computational modeling may enhance mechanistic insights and contribute to evolving standards for preclinical validation. Ultimately, these systems could play a meaningful role in the biomedical industry, helping to enable more predictive, personalized, and ethically responsible drug development pipelines, while supporting further progress in regenerative medicine and precision cardiology.

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Figure 5. The authors recreated the figure in an editable format, verified all content for accuracy, and take full responsibility for it. We thank Dr. J. Peter Van Tintelen for granting permission to adapt and use a modified version of Figure 1.

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Conflict of interest

The authors declare they have no competing interests.

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