Induced Pluripotent Stem Cell–Derived Cardiomyocytes in Drug Development: Model Construction, Applications, And Future Perspectives

Xinliang Zhao *

Department of Biological Engineering, Southwest Jiaotong University, Sichuan, 610000, China

* Corresponding Author Email: XinliangZhao@my.swjtu.edu.cn

Abstract. Drug-induced cardiotoxicity remains one of the major causes of failure or withdrawal of candidate compounds in development. Human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) offer a promising human-relevant in vitro platform to assess cardiac safety, enabling disease modeling, patient-specific studies, and high-throughput screening. This research summarizes current strategies for constructing iPSC-CM models, including differentiation protocols and maturation methods; this research outlines the general workflow of cardiotoxicity testing and describe in detail key functional assessments: electrophysiological, contractility, structural, metabolic, and population-level. This research also discusses the advantages and the remaining challenges (e.g. immaturity, phenotypic variability, cost, lack of standardization) and highlight possible solutions. Combined maturation strategies including long-term culture with mechanical and electrical stimulation have improved outcomes. Collaborative consortia are also increasingly promoting the process for eventual regulatory acceptance. This research aims to present future perspectives for improving predictive power and facilitating translation into clinical applications.

Keywords: iPSC-CM; cardiotoxicity; electrophysiology; high-throughput screening.

1. Introduction

Cardiotoxicity is a critical safety concern in both drug discovery and clinical use. Many promising pharmacological agents are discontinued either during preclinical phases or after market approval due to unexpected adverse cardiac events such as arrhythmia, cardiomyopathy or QT-interval prolongation. Traditional models—animal studies, immortalized cell lines, and primary human cardiomyocytes—have provided insight, but each has limitations. Animal models do not always replicate human ionic currents or genetic background; immortalized cell lines often lack the full complement of cardiac-specific ion channels and structural features; while primary cardiomyocytes are scarce, difficult to maintain, and have limited proliferative capacity. The development of induced pluripotent stem cell (iPSC) technology has opened a new avenue. Somatic cells reprogrammed to pluripotency can be differentiated into cardiomyocytes (iPSC-CMs). These cells retain human genomic context, can be generated from different individuals (including patients with genetic cardiac disease), and afford the possibility of scalable in vitro assays. Over the past decade, iPSC-CMs have been leveraged for disease modeling, drug testing, and safety pharmacology [1-3].

However, there are gaps: iPSC-CMs often show immature electrophysiological, structural, and metabolic phenotypes relative to adult cardiomyocytes. In addition, standardization of protocols, reproducibility among cell lines/labs, throughput, and cost are issues. This review aims to (1) describe current methods for model construction and maturation. (2) detail the workflow of cardiotoxicity testing with emphasis on major functional assessments. (3) analyze advantages vs. challenges, and (4) propose future directions to improve utility in drug development.

2. iPSC-CM Model Construction

2.1. Overview of Differentiation

Generation of iPSC-CMs typically starts from reprogramming somatic cells into iPSCs, then differentiating them toward the cardiac lineage. Differentiation protocols rely on modulating developmental signaling pathways, especially Wnt/β-catenin, Activin/Nodal, and BMP, to drive mesoderm induction followed by cardiac specification [4]. Commercial differentiation kits simplify the procedure and provide more standardized reagents, but custom protocols often allow adjustments to optimize yield, purity, or align with particular assessment methods. Key parameters include the timing and concentration of signaling modulators, cell density, substrate or matrix composition, and selection or enrichment of cardiomyocytes via e.g. metabolic selection or reporter lines.

2.2. Rationale for Maturation

Although iPSC-CMs possess clear advantages in terms of human origin and scalability, their immature phenotype significantly limits their utility in drug research. Compared with adult cardiomyocytes, iPSC-CMs display notable differences in action potential duration, contractile force, calcium handling, and energy metabolism. These discrepancies may lead to underestimation or overestimation of drug effects, particularly in the evaluation of arrhythmogenic risk or metabolism-related toxicity. Therefore, achieving structural and functional maturation is a critical step toward enhancing the predictive accuracy and clinical relevance of iPSC-CM models.

2.3. Maturation Strategies

To overcome immaturity, a variety of maturation methods have been developed: Long-term culture: Extended in vitro culture improves sarcomere organization, increases expression of mature isoforms of contractile proteins and ion channels, and improves calcium transient kinetics [4]. Electrical stimulation: Applying pacing or electrical field stimulation enhances action potential profiles and conduction properties [3]. Mechanical stimulation / substrate engineering: Using matrices of controlled stiffness, cyclic stretch or scaffolds mimicking mechanical load promotes alignment and contractility [5]. 3D culture and engineered tissues: Engineered heart tissues (EHTs), cardiac spheroids, or organoids allow 3D interactions and improve maturation [3]. Biochemical cues: Hormones (thyroid hormone, glucocorticoids), metabolic substrate switching, and mitochondrial enhancers support metabolic maturation [4]. Combination approaches generally yield better maturation outcomes than single methods.

3. Applications of iPSC-CM in Drug Development

In modern drug development, cardiotoxicity assessment has moved beyond a singular focus on hERG assays and QT prolongation to more integrative approaches. The FDA's Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative emphasizes the use of multiple ionic current recordings, in silico modeling, and human-derived cardiomyocyte assays, highlighting the role of iPSC-CMs as an advanced testing platform [6]. Unlike immortalized cell lines or animal models, iPSC-CMs express a wide repertoire of cardiac ion channels, contractile proteins, and metabolic machinery, which makes them well-suited to predict complex cardiotoxic mechanisms. The general workflow begins with candidate compound exposure to iPSC-CM monolayers or 3D engineered heart tissues (EHTs). Functional data are collected across multiple dimensions: electrophysiology, contractility, sarcomere and mitochondrial integrity, metabolic flux, and donor-specific variability. These data are then integrated into safety profiles and guide the decision whether a compound proceeds to animal or clinical testing.

3.1. Electrophysiological Assessment

Electrophysiology remains the cornerstone of cardiac safety testing. Patch clamp recording is the gold standard for measuring specific ionic currents, but it is labor-intensive and low-throughput. MEA (multi-electrode array) technology addresses this by enabling parallel measurements of extracellular field potentials across thousands of cells. Importantly, iPSC-CM assays can recapitulate known drug responses. For instance, cisapride and terfenadine, both withdrawn from the market due to arrhythmogenic side effects, induce prolonged field potential duration in iPSC-CMs, consistent with QT prolongation observed in patients [1, 5]. Other examples include dofetilide and sotalol, which prolong repolarization, while nifedipine shortens it through calcium channel blockade. Recent studies also highlight the advantage of combining MEA with voltage-sensitive dyes or calcium imaging, providing multiparametric readouts that improve predictive accuracy [7]. Compared to the traditional hERG assay, iPSC-CM platforms capture not only repolarization abnormalities but also triggered activity, conduction slowing, and arrhythmogenic events such as early afterdepolarizations (EADs). This integrative readout aligns well with CiPA's emphasis on holistic evaluation [6].

3.2. Contractility Assessment

Cardiomyocyte contraction is a fundamental endpoint for drug safety and efficacy. iPSC-CM contractility assays can be performed on 2D monolayers, where motion-tracking software quantifies beating frequency, amplitude, and velocity. Alternatively, 3D EHTs provide force measurements, better mimicking myocardial tissue. iPSC-CMs reproduce the expected inotropic effects of known drugs. For instance, isoproterenol, a β-adrenergic agonist, increases beat frequency and contraction amplitude, while β-blockers such as propranolol reduce them [2]. Negative inotropes like verapamil depress contraction force, consistent with clinical effects. The use of traction force microscopy further enables single-cell resolution, identifying heterogeneity among cardiomyocytes. EHTs, meanwhile, provide tissue-level readouts such as twitch force and relaxation kinetics. Such assays not only predict toxicity but also serve in efficacy testing for drugs intended to improve contractility, e.g., myosin activators for heart failure.

3.3. Structural Toxicity Assessment

Structural toxicity reflects chronic drug exposure effects, manifesting as sarcomere disarray, nuclear fragmentation, or cytoskeletal collapse. Anthracycline chemotherapeutics such as doxorubicin are prototypical examples. iPSC-CMs exposed to doxorubicin show disrupted sarcomere organization, mitochondrial swelling, and increased apoptosis, paralleling patient cardiomyopathy [2, 3]. High-content imaging (HCI) systems combined with immunofluorescence staining (e.g., α-actinin for sarcomeres, DAPI for nuclei) enable automated quantification of structural damage at scale. This high-throughput compatibility makes iPSC-CMs suitable for screening compound libraries. Another example is trastuzumab (Herceptin), a monoclonal antibody that can induce cardiotoxicity in patients. iPSC-CM assays detect reduced contractile protein expression and compromised mitochondrial activity following trastuzumab treatment, providing early mechanistic insight [4].

3.4. Metabolic Function Assessment

Cardiomyocytes are highly energy-demanding cells, and metabolic impairment is a common mechanism of cardiotoxicity. Immature iPSC-CMs initially rely on glycolysis, but maturation strategies enable a shift toward fatty acid oxidation, closer to adult metabolism. Functional assays such as Seahorse XF analyzers measure oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), offering insights into oxidative phosphorylation versus glycolysis balance [8]. Using these methods, researchers have shown that tyrosine kinase inhibitors such as imatinib disrupt mitochondrial respiration, while anthracyclines impair ATP production. iPSC-CMs also allow identification of mitochondrial permeability transition pore opening, membrane depolarization, and reactive oxygen species (ROS) accumulation. Such assays are essential to capture toxicity beyond electrophysiological disturbances, highlighting the value of multi-dimensional assessments [1].

3.5. Population-Level Assessment

One of the most powerful aspects of iPSC technology is the ability to generate cardiomyocytes from diverse donors. This enables modeling of population-level drug responses, reflecting genetic variability. Disease-specific iPSC-CMs provide unique insights. For example, iPSC-CMs derived from long QT syndrome (LQTS) patients display prolonged repolarization, which can be normalized by β-blockers [8]. Similarly, catecholaminergic polymorphic ventricular tachycardia (CPVT) lines exhibit abnormal calcium handling that predisposes to arrhythmia under adrenergic stress. Beyond natural variability, CRISPR/Cas9 genome editing allows the creation of isogenic pairs differing only at specific mutations, enabling precise attribution of phenotypic effects [4]. This makes iPSC-CM platforms valuable not only for drug safety but also for precision medicine, where therapies can be tailored to individual genetic backgrounds.

4. Discussion and Future Exploration

The iPSC-CM model presents several key advantages that make it a valuable tool in drug development. Its foremost strength is the human origin of the cells, which ensures the presence of the full complement of cardiac ion channels, receptors, and contractile proteins typically found in human myocardium. This makes iPSC-CMs more predictive of clinical outcomes than traditional animal models, which often diverge in electrophysiological properties and drug sensitivities. Another notable advantage is the potential for patient- and disease-specific modeling. iPSCs can be derived from individuals with inherited cardiac conditions, such as long QT syndrome or hypertrophic cardiomyopathy, thereby providing a unique platform for precision medicine. In terms of scalability and technical compatibility, iPSC-CMs can be generated in large quantities and are amenable to integration with high-throughput platforms such as multi-electrode arrays, high-content imaging, and metabolic flux assays. This adaptability facilitates their use in pharmaceutical pipelines for large-scale compound screening and mechanistic toxicology studies. Furthermore, the renewable nature of iPSCs reduces dependence on animal experiments, which not only addresses ethical concerns but also improves translational relevance by directly using human cells. Nevertheless, several significant challenges remain. A major limitation is the immature phenotype of iPSC-CMs. They typically exhibit reduced inward rectifier potassium current (I_K1), a depolarized resting membrane potential, and underdeveloped metabolic pathways, which collectively compromise their ability to fully replicate adult cardiomyocyte physiology. This immaturity can obscure or distort drug responses, leading to potential underestimation of adverse effects. Variability between iPSC lines, donor backgrounds, and differentiation batches adds another layer of complexity. High culture costs and the lack of universally accepted maturity benchmarks further complicate implementation.

Overcoming these challenges requires multifaceted approaches. Combined maturation strategies such as long-term culture with mechanical and electrical stimulation have improved electrophysiological and metabolic outcomes. Genome editing with CRISPR/Cas9 enables isogenic controls to reduce variability. Machine learning and artificial intelligence are increasingly applied to extract robust patterns from high-dimensional assay data, improving interpretation [9]. Finally, international collaboration is essential for establishing standardized protocols and regulatory guidelines.In summary, iPSC-CMs provide an unprecedented opportunity to advance human-relevant drug safety testing, but immaturity, variability, and cost remain significant barriers. Continued innovation in maturation methods, computational tools, and standardization will be critical to realizing their full potential as a cornerstone in cardiotoxicity assessment. In the near future, continued refinement of differentiation and maturation protocols will address immaturity, improving predictive accuracy. Advances in 3D culture systems, engineered heart tissues, and organoids are likely to provide more physiologically relevant readouts. At the same time, integration of multiparametric assays into medium- and high-throughput platforms will enable broader adoption in pharmaceutical pipelines. Over the next 5–10 years, researchers anticipate broader integration of computational tools. Artificial intelligence (AI) and machine learning will play a central role in

analyzing the vast datasets generated from imaging, MEA, and metabolic assays [9]. Coupled with in silico cardiac modeling, these approaches may allow virtual trials that simulate population variability and predict rare adverse events. Furthermore, efforts toward standardization—common protocols, validated endpoints, and quality control measures—will help harmonize data across laboratories. Collaborative consortia and regulatory bodies (e.g., FDA, EMA) are increasingly engaging with iPSC-CM platforms, paving the way for eventual regulatory acceptance [6].

5. Conclusion

The past decade has seen remarkable advances in the use of iPSC-CMs for cardiotoxicity assessment and drug development. By combining electrophysiological, contractile, structural, metabolic, and population-level analyses, researchers can now build multidimensional profiles of drug action. These insights complement traditional assays and animal studies, offering human-relevant data earlier in the drug pipeline. Looking further ahead, iPSC-CMs may be incorporated into personalized medicine frameworks. Integration with electronic health records and genomic data could allow patient-specific drug testing, predicting adverse effects before therapy is initiated. Gene editing could be applied to correct pathogenic variants, enabling "disease-in-a-dish" models for therapeutic discovery. On a global scale, reducing reliance on animal studies through validated human iPSC-CM platforms aligns with the 3R principles (replacement, reduction, refinement). Ultimately, this research envision a future where iPSC-CM assays, combined with computational modeling and population-scale variability, become a gold standard in drug development and safety pharmacology.

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