J Physiol 602.20 (2024) pp 5155–5162

REVIEW-SYMPOSIUM

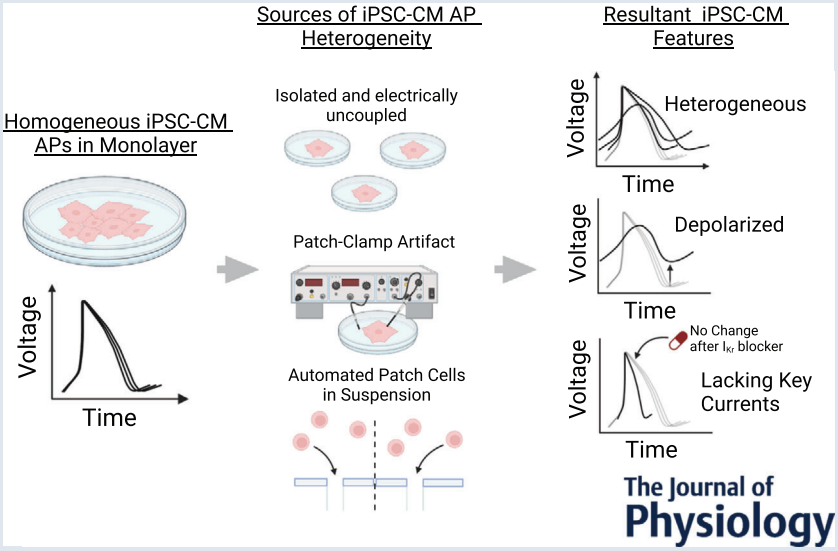
Stem cell-derived cardiomyocyte heterogeneity confounds electrophysiological insights

Alexander P. Clark[1], Trine Krogh-Madsen[2,3] and David J. Christini[1,4]

[1]*Department of Biomedical Engineering, Cornell University, Ithaca, NY, USA*
[2]*Department of Physiology & Biophysics, Weill Cornell Medicine, New York, NY, USA*
[3]*Institute for Computational Biomedicine, Weill Cornell Medicine, New York, NY, USA*
[4]*Department of Physiology and Pharmacology, SUNY Downstate Health Sciences University, Brooklyn, NY, USA*

Handling Editors: Laura Bennet & Eleonora Grandi

The peer review history is available in the Supporting Information section of this article (https://doi.org/10.1113/JP284618#support-information-section).



Abstract Human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) offer potential as an *in vitro* model for studying drug cardiotoxicity and patient-specific cardiovascular disease. The inherent electrophysiological heterogeneity of these cells limits the depth of insights that can be drawn from well-designed experiments. In this review, we provide our perspective on some sources and the consequences of iPSC-CM heterogeneity. We demonstrate the extent of heterogeneity in the literature and explain how such heterogeneity is exacerbated by patch-clamp experimental artifacts in the manual and automated set-up. Finally, we discuss how

Alex Clark completed his doctoral training in biomedical engineering at Cornell University (Ithaca, NY, USA) with David Christini. While in graduate school, Alex became interested in the heterogeneity and emergent properties of cardiomyocyte action potentials. He took a systems approach and used computational and experimental stem cell-derived cardiomyocyte models to study endogenous and exogenous factors affecting cardiomyocyte electrophysiology. Currently, he is a postdoctoral fellow in the Cardiac Systems Biology group at the University of Virginia, mentored by Jeffrey Saucerman. He is interested in using single-cell sequencing data to map the gene regulatory networks required to specify and maintain cardiomyocyte identity.

DOI: 10.1113/JP284618

The Journal of Physiology

this heterogeneity, caused by both intrinsic and extrinsic factors, limits our ability to build digital twins of patient-derived cardiomyocytes.

(Received 31 January 2024; accepted after revision 24 April 2024; first published online 9 May 2024)
Corresponding author D. J. Christini: Department of Physiology and Pharmacology, SUNY Downstate Health Sciences University, Brooklyn, NY 11203 USA. Email: David.Christini@downstate.edu

Abstract figure legend Human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) offer potential as an *in vitro* model for studying drug cardiotoxicity and patient-specific cardiovascular disease. However, the electrophysiological heterogeneity of these cells confounds experimental results and limits reproducibility. This limitation is due to both exogenous and endogenous variables that can be very difficult to parse during experiments. In this review, we discuss sources of iPSC-CM heterogeneity, our mechanistic understanding of these contributors, and how we think about studying and addressing these issues.

Introduction

Human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) are a widely available cell type used in both academia and industry. These cells have been particularly useful to study proarrhythmic substrates originating at the cellular level, including genetic

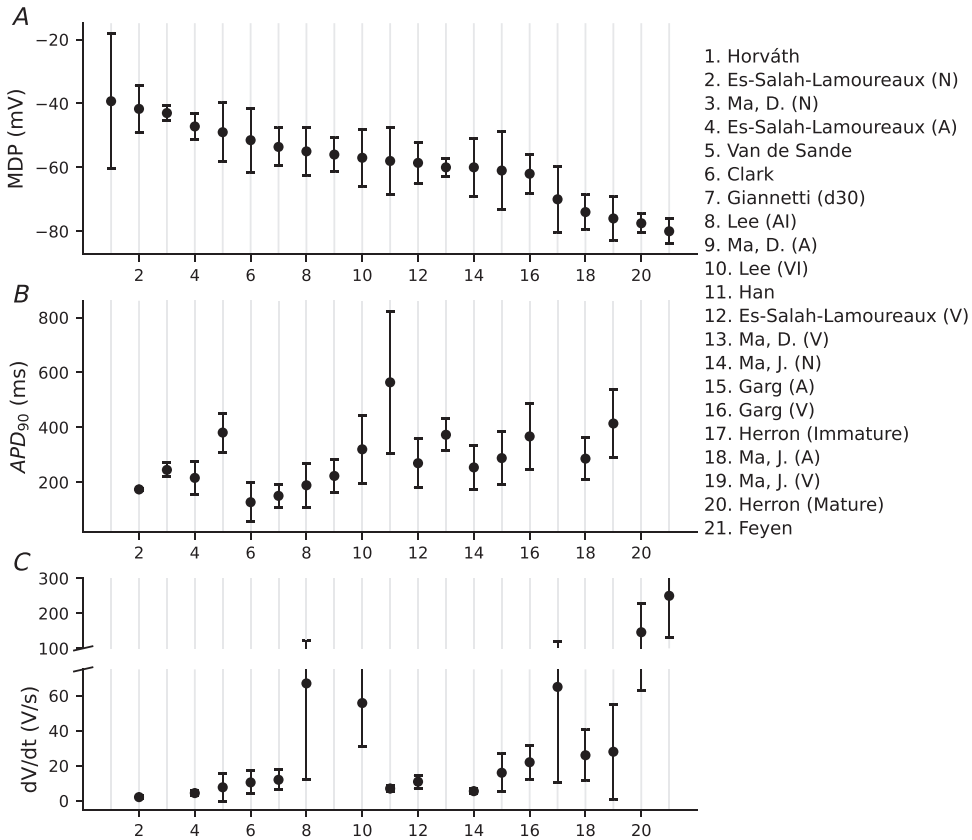


Figure 1. Inter- and intralab AP feature heterogeneity
Mean and standard deviations of action potential MDP, APD_{90} and dV/dt_{max} from 21 independent datasets. These datasets were taken from 12 different studies, where APs were recorded without the injection of hyperpolarizing current: (Clark et al., 2022; EsSalah-Lamoureux et al., 2016; Feyen et al., 2020; Garg et al., 2019; Giannetti et al., 2021; Han et al., 2014; Herron et al., 2016; Horváth et al., 2018; Lee et al., 2017; Ma et al., 2015; Ma et al., 2011; Van de Sande et al., 2021). Studies with 'N', 'A' and 'V' indicate that these cells were sorted into nodal, atrial or ventricular categories based on AP morphology. 'AI' and 'VI' indicate that these data are from a study that compared iPSC-CMs derived from either an atrial or a ventricular differentiation protocol. The 'd30' next to Giannetti et al. (2021) indicates that these features are from a group of cells differentiated to 30 days, as other time points were also considered in the study. The iPSC-CMs from all other studies were not categorized.

mutations (Han et al., 2014; Sala et al., 2019) and drugs that block cardiac ion channels (Liang et al., 2013). While many studies have shown differences between control and experimental groups, the depth of insights from iPSC-CM data and the ability to reproduce experiments is limited by their electrophysiological immaturity (Goversen et al., 2018) and inter-/intralab heterogeneity (Blinova et al., 2018; Prajapati et al., 2021). In Fig. 1, we plot the mean ± SD of human iPSC-CM spontaneous action potential (AP) features for 21 independent datasets taken from 12 studies – these features include maximum diastolic potential (MDP), action potential duration at 90% repolarization (APD_{90}), and maximum upstroke velocity (dV/dt_{max}). There was no hyperpolarizing current injected into the cells during any of these studies, a practice that has been used with both adult human (Workman et al., 2006) and iPSC-derived cardiomyocytes (Quach et al., 2018) during patch-clamp experiments. The depolarized MDP of the cells in Fig. 1 likely contributes to a reduced dV/dt_{max} and affects the APD_{90} in most datasets. These data illustrate the extent of inter- and intralab heterogeneity present in iPSC-CM studies. The inherent electrophysiological variation of these cells makes it difficult to measure consistent population-level signals, and often confounds experimental results. In this review, we discuss our thoughts on iPSC-CM electrophysiological heterogeneity, from intrinsic/extrinsic sources to its consequences for precision medicine.

iPSC-CM heterogeneity confounds experimental results and limits reproducibility

Shortly after the development of iPSC-CMs (Zhang et al., 2009), investigators began to sort cells into nodal, atrial and ventricular groups based on their AP features (Itzhaki et al., 2011; Ma et al., 2011; Moretti et al., 2010). Resultant sub-groups have less variance in AP morphology than the population as a whole; i.e. sub-grouping reduces the apparent population heterogeneity. It has since been shown that, when groupings are ignored, iPSC-CM AP features are normally distributed (Du et al., 2015), and categorization of iPSC-CMs into heart chamber (or nodal) groups can be misleading and reductive. For example, individual iPSC-CMs do not always have canonical gene expression profiles and can be sensitive to non-canonical modulators of cell fate, pointing towards a physiology that is not seen in adult primary cardiomyocytes (Biendarra-Tiegs et al., 2019). Similarly, gene expression and phenotype data acquired from iPSC-CMs has shown a combination of attributes in a mixed population that is not consistent with any one chamber (Schmid et al., 2021).

iPSC-CM heterogeneity has long been viewed as a problem that begets research into differentiation protocol optimization for the purpose of producing consistent, largely homogeneous cellular populations for *in vitro* cardiotoxicity studies (Blinova et al., 2018). Commercial cell lines produced to limit heterogeneity have succeeded in producing consistent AP features with little batch-to-batch variability when recorded from coupled monolayers (Hayes et al., 2019). These lines, however, show greater heterogeneity when electrically isolated (López-Redondo et al., 2016), a characteristic also seen with isolated human adult cardiomyocytes (Verkerk et al., 2017). We believe these characteristics of iPSC-CMs should be reframed as a biological reality that serves as a laboratory model of cardiomyocyte heterogeneity in the adult context.

It is well known that the AP morphology of primary cardiomyocytes isolated from the same regions of the heart can vary widely (Lachaud et al., 2022; Zaniboni et al., 2000). Lachaud et al. (2022) isolated 150 cells from a single rabbit left ventricle and, using optical recordings, showed that the APD_{90} ranged from 100 to >300 ms. While these primary cells specifically did not possess the multi-chamber molecular expression heterogeneity (Moretti et al., 2010) seen in iPSC-CMs, such results still indicate that substantial cell-to-cell variation in AP morphology is ubiquitous. iPSC-CMs have the potential to serve as a valuable tool to study and develop methods to help us understand electrophysiological heterogeneity with the hope of surfacing physiologically relevant patterns.

Automated patch-clamp systems exacerbate non-physiological state of iPSC-CMs

Automated patch clamp (APC) systems provide a tantalizing solution to the question: how can the throughput of single-cell iPSC-CM patch clamp studies be increased? Unfortunately, the list of iPSC-CM shortcomings only grows when they are used in an automated system. For example, iPSC-CMs are very depolarized in the APC system (e.g. >−15 mV in our accompanying paper), requiring the injection of a constant hyperpolarizing current in addition to I_{K1} and seal-leak dynamic clamp to maintain a resting membrane potential (Becker et al., 2020). Large hyperpolarizing currents are also required to maintain a resting membrane potential in primary pig cardiomyocytes tested in APC (Seibertz et al., 2022). In our accompanying manuscript, our data indicate that I_{Kr} is not present at measurable levels during APC experiments at physiological concentrations. Another review stated that there are no studies showing delayed rectifier potassium currents in iPSC-CM data measured using an APC (Ismaili et al., 2023); however, a very recent study showed that replacing K^+ ions with Cs^+ ions can be used to uncover a sizable I_{Kr} (Bloothooft et al., 2024). All of this points to the APC set-up causing a

substantial deviation of iPSC-CM electrophysiology from cell-attached set-ups. We hypothesize that this is caused by the need for cells to be dissociated and in suspension when conducting such experiments. This leads us to ask the following two questions:

- *Can iPSC-CMs be produced that consistently have a maximum diastolic potential <−75 mV without the addition of any hyperpolarizing currents?* The cells from our paper were quiescent and had a minimum potential of >−15 mV, which is greater than even the most depolarized cells from manual patch experiments from our lab (see Fig. 1, Clark et al., 2022). It is important to understand which ionic currents are altered to cause such a different phenotype while in the APC set-up.
- *Why is I_{Kr} present and measurable during manual experiments, but not during automated?* As mentioned above, this seems likely to pertain to the cellular preparation – either the dissociation agent causes a reduction in functional transmembrane I_{Kr} channels or I_{Kr} channels are not trafficked or expressed in cardiomyocytes when cells are in suspension.

This is not an exhaustive list, but these issues must be addressed before the APC can be used at scale for cardiotoxicity screening. We believe that solving them has far-reaching implications. Having a good iPSC-CM model for automated systems would make it possible for a 10−100× increase in output, with a fraction of the effort.

iPSC-CMs are susceptible to experimental artifact that increases electrophysiological heterogeneity

After digging into recently published experimental artifact models (Lei et al., 2020), probing our own *in vitro* data, and investigating interlab AP heterogeneity, we have come to believe that experimental artifact contributes substantially to iPSC-CM AP morphology in much of the literature. An imperfect seal between the pipette tip and iPSC-CM cell membrane is a likely source of non-trivial depolarization in the maximum diastolic potential of these cells during patch-clamp experiments (Clark et al., 2023; Horváth et al., 2018; Van de Sande et al., 2021). Due to their small size and low I_{K1} density, iPSC-CMs are particularly susceptible (compared to adult primary cardiomyocytes) to seal-leak contributions. Additionally, this extrinsic artifact is not easily measured, nor can it be easily corrected due to its susceptibility to change throughout an experiment. As such, seal-leak is likely a pervasive issue in the iPSC-CM AP literature. The most direct way to compensate for this artifact during current-clamp recordings is through seal-leak dynamic clamp compensation that can return the iPSC-CM action potential to a morphology more like its unpatched state. When seal-leak compensation is coupled with I_{K1} dynamic clamp, iPSC-CM APs can have a morphology similar to adult-like cardiomyocytes and may provide a better model for pharmacological studies (Verkerk & Wilders, 2021). Furthermore, seal-leak current should not be ignored when constructing iPSC-CM electrophysiological models that are fit to published AP data.

Recent work from Lei et al. (2020) has also shown both (1) the confounding effects of patch-clamp artifacts in voltage clamp experiments, and (2) the descriptive value of including experimental artifacts when modelling single-cell electrophysiology. While it is well-known that fast currents, like I_{Na}, are highly sensitive to experimental artifact (e.g. series resistance), Lei et al. (2020) shows that smaller (in density) and slower (in kinetics) currents, like I_{Kr}, can also be substantially altered by such artifact. Amplifiers are designed to correct for these effects, but that compensation can be insufficient when studies are designed to investigate ion channel kinetics. As such, artifact equations provide a means to augment the descriptive power of electrophysiological models, especially iPSC-CM models (Kernik et al., 2019; Paci et al., 2018), when interpreting and simulating *in vitro* experiments (Clark et al., 2022).

In the future, we recommend computational models be developed with equations for both single-cell ionic current kinetics and patch-clamp artifacts. Such fully-integrated iPSC-CM+artifact models would be the most descriptive, to date, of these cells within the experimental context in which the majority of data is acquired.

iPSC-CM heterogeneity stymies efforts to develop patient-specific cardiomyocyte digital twins

A digital twin is a computational, often mechanistic model (or set of models) that is constructed to reproduce patient-specific features. The model can be used to investigate a patient's unique pathophysiology and design a targeted therapeutic strategy – the potential of this approach in precision cardiology has been extensively covered in a recent review (Corral-Acero et al., 2020). Our lab has previously taken steps in the direction of developing cellular digital twins by constructing cell-specific AP models of guinea pig cardiomyocytes (Groenendaal et al., 2015). The ability of these computational models to reproduce complex *in vitro* data indicates that it may be possible to develop human cell-specific models, and more specifically, computational models of patient-derived iPSC-CMs with cell-specific conductance parameters. One could use such cell-specific models to quantify and understand inter-cell heterogeneity in AP features and response to drug treatment. Our attempts at following through on this have not worked – in short, fitted iPSC-CM models have

Table 1. A summary of challenges and considerations for conducting and analysing iPSC-CM patch-clamp experiments

Challenges	Considerations
iPSC-CMs have a depolarized MDP	Dynamically clamp I_{K1} and seal-leak compensation current
Repolarizing potassium current (including I_{Kr}) density appears to be reduced during APC studies	Dynamically clamp I_{K1}/seal-leak compensation current and inject hyperpolarizing current. Use a Cs^+ extracellular solution if recording I_{Kr} current (Bloothooft et al., 2024).
iPSC-CM computational models do not reproduce the effects of experimental artifact on AP morphology	Incorporate experimental artifact equations into iPSCCM models.
iPSC-CM computational models cannot reproduce all the dynamics of experimental data	Focus on using model fits to predict responses to realistic perturbations.

been incapable of reproducing the ionic current dynamics of the *in vitro* data.

We believe the fitting failed to produce meaningful patient-specific models for two main reasons.

First, the computational models (Kernik et al., 2019; Paci et al., 2018) may not be representative of the cells that were fitted. The Kernik et al. (2019) model was fit to data from several studies, while Paci et al. (2018) is based primarily on the data from Ma et al. (2011) – the cell lines used to fit these models were not included in our *in vitro* studies. While these models provide meaningful descriptive capacity, it seems they do not provide a suitable starting point for fitting the cell-specific dynamics of the *in vitro* data from our lab.

Second, many fitted iPSC-CM models are unlikely to produce good estimates for all parameters of interest – this is because most models are sloppy, as defined in Gutenkunst et al. (2007), meaning that fits to experimental data often result in poorly constrained parameters. Gutenkunst et al. (2007) make the following statement about sloppy models:

> Concrete predictions can be extracted from models long before their parameters are even roughly known, and, when a system is not already well-understood, it can be more profitable to design experiments to directly improve predictions of interesting system behaviour rather than to improve estimates of parameters.

Given the AP heterogeneity seen from lab-to-lab and the inability to produce models that adequately reproduce complex current dynamics, we would argue that the iPSC-CM electrophysiological system is not well-understood. One approach that focuses on prediction and addresses the shortcomings that arise from experimental heterogeneity and sloppy models is to generate a population of plausible parameter sets that produce simulations constrained to a range of experimental AP and/or calcium transient data (Paci et al., 2020, 2021). Sets of models generated in this way can provide robust predictions of population responses to a perturbation (e.g. drug treatment) and do not always rely on accurate estimation of parameter values or single-channel dynamics.

While the development of cell-specific models has enormous potential, it is clearly very difficult. If one plans to pursue a cell-specific modelling project with iPSC-CMs, we recommend considering the following before starting:

- *How good a fit is good enough?* A model is imperfect by definition so a clear success criterion should be established *a priori*. Is the goal to reproduce certain validation data or predict parameters? Of course, the answer may be both, but realistically, these are two very different goals and will influence how to design and assess a model. If the fitted model fails to achieve the goal, we have found it fruitful to quantify and attempt to explain discrepancies between models and the *in vitro* data – we believe reporting this is valuable to the field. Additionally, it may make sense to start by fitting a cell-line-specific model for iPSC-CMs from each donor before then attempting a cell-specific model.
- *What is the purpose of developing the model?* Cell-specific modelling is recognized as a solution to numerous problems. Before starting a project, we recommend either: (1) identifying a specific problem one is trying to address with cell-specific modelling, or (2) enumerating the problems that cell-specific modelling will help address. These can anchor the project if the cell-specific modelling does not work out.

Answering these questions and being realistic about the potential of models to reproduce AP behaviour are essential to scoping a tractable project.

iPSC-CM heterogeneity needs to be studied

iPSC-CM heterogeneity has long been characterized as a problem that needs addressing. In this review, we provide details on the technical challenges of studying iPSC-CM electrophysiology and provide suggestions for how to deal

with some of these issues (summarized in Table 1). We also attempt to reframe heterogeneity as a physiological reality that should be studied. There is value (especially in the drug screening context) in developing iPSC-CM lines with homogeneous ionic current expression levels and that produce consistent results. But there is also value in using iPSC-CMs as a model to study heterogeneity. A big part of this heterogeneity research must focus on differences from cell-to-cell, *vs*. batch-to-batch, *vs*. lab-to-lab, *vs*. patient-to-patient. Understanding how each of these sources contributes to the iPSC-CM electrophysiological phenotype has important implications from basic science research to the use of iPSC-CMs as a therapeutic tool and model for drug screening.

References

Becker, N., Horváth, A., De Boer, T., Fabbri, A., Grad, C., Fertig, N., George, M., & Obergrussberger, A. (2020). Automated dynamic clamp for simulation of IK1 in human induced pluripotent stem cell–derived cardiomyocytes in real time using patchliner dynamite[8]. *Current Protocols in Pharmacology*, **88**(1), e70.

Biendarra-Tiegs, S. M., Li, X., Ye, D., Brandt, E. B., Ackerman, M. J., & Nelson, T. J. (2019). Single-cell RNA-sequencing and optical electrophysiology of human induced pluripotent stem cell-derived cardiomyocytes reveal discordance between cardiac subtype-associated gene expression patterns and electrophysiological phenotypes. *Stem Cells and Development*, **28**(10), 659–673.

Blinova, K., Dang, Q., Millard, D., Smith, G., Pierson, J., Guo, L., Brock, M., Lu, H. R., Kraushaar, U., Zeng, H., Shi, H., Zhang, X., Sawada, K., Osada, T., Kanda, Y., Sekino, Y., Pang, L.i, Feaster, T. K., Kettenhofen, R., Stockbridge, N., Strauss, D. G., & Gintant, G. (2018). International multisite study of human-induced pluripotent stem cell-derived cardiomyocytes for drug proarrhythmic potential assessment. *Cell Reports*, **24**(13), 3582–3592.

Bloothooft, M., Verbruggen, B., Seibertz, F., Heyden, M. A. G. V. D., Voigt, N., & De Boer, T. P. (2024). Recording ten-fold larger IKr conductances with automated patch clamping using equimolar Cs+ solutions. *Frontiers in Physiology*, **15**, 1298340.

Clark, A. P., Clerx, M., Wei, S., Lei, C. L., De Boer, T. P., Mirams, G. R., Christini, D. J., & Krogh-Madsen, T. (2023). Leak current, even with gigaohm seals, can cause misinterpretation of stem cell-derived cardiomyocyte action potential recordings. *Europace*, **25**(9), euad243.

Clark, A. P., Wei, S., Kalola, D., Krogh-Madsen, T., & Christini, D. J. (2022). An in silico-in vitro pipeline for drug cardiotoxicity screening identifies ionic pro-arrhythmia mechanisms. *British Journal of Pharmacology*, **179**(20), 4829–4843.

Corral-Acero, J., Margara, F., Marciniak, M., Rodero, C., Loncaric, F., Feng, Y., Gilbert, A., Fernandes, J. F., Bukhari, H. A., Wajdan, A., Martinez, M. V., Santos, M. S., Shamohammdi, M., Luo, H., Westphal, P., Leeson, P., Diachille, P., Gurev, V., Mayr, M., Geris, L., Pathmanathan, P., Morrison, T., Cornelussen, R., Prinzen, F., Delhaas, T., Doltra, A., Sitges, M., Vigmond, E. J., Zacur, E., Grau, V., Rodriguez, B., Remme, E. W., Niederer, S., Mortier, P., Mcleod, K., Potse, M., Pueyo, E., Bueno-Orovio, A., & Lamata, P. (2020). The 'Digital Twin' to enable the vision of precision cardiology. *European Heart Journal*, **41**(48), 4556–4564.

Du, D. T. M., Hellen, N., Kane, C., & Terracciano, C. M. N. (2015). Action potential morphology of human induced pluripotent stem cell-derived cardiomyocytes does not predict cardiac chamber specificity and is dependent on cell density. *Biophysical Journal*, **108**(1), 1–4.

Es-Salah-Lamoureux, Z., Jouni, M., Malak, O. A., Belbachir, N., Al Sayed, Z. R., Gandon-Renard, M., Lamirault, G., Gauthier, C., Baró, I., Charpentier, F., Zibara, K., Lemarchand, P., Beaumelle, B., Gaborit, N., & Loussouarn, G. (2016). HIV-Tat induces a decrease in IKr and IKs via reduction in phosphatidylinositol-(4,5)-bisphosphate availability. *Journal of Molecular and Cellular Cardiology*, **99**, 1–13.

Feyen, D. A., McKeithan, W. L., Bruyneel, A. A., Spiering, S., Hörmann, L., Ulmer, B., Zhang, H., Briganti, F., Schweizer, M., Hegyi, B., Liao, Z., P¨ol¨onen, R. P., Ginsburg, K. S., Lam, C. K., Serrano, R., Wahlquist, C., Kreymerman, A., Vu, M., Amatya, P. L., Behrens, C. S., Ranjbarvaziri, S., Maas, R. G., Greenhaw, M., Bernstein, D., Wu, J. C., Bers, D. M., Eschenhagen, T., Metallo, C. M., & Mercola, M. (2020). Metabolic maturation media improve physiological function of human iPSC-derived cardiomyocytes. *Cell Reports*, **32**(3), 107925.

Garg, P., Oikonomopoulos, A., Chen, H., Li, Y., Lam, C. K., Sallam, K., Perez, M., Lux, R. L., Sanguinetti, M. C., & Wu, J. C. (2019). Genome editing and induced pluripotent stem cells in cardiac channelopathy. *Journal of the American College of Cardiology*, **72**(1), 62–75.

Giannetti, F., Benzoni, P., Campostrini, G., Milanesi, R., Bucchi, A., Baruscotti, M., Dell'era, P., Rossini, A., & Barbuti, A. (2021). A detailed characterization of the hyperpolarization-activated "funny" current (If) in human-induced pluripotent stem cell (iPSC)-derived cardiomyocytes with pacemaker activity. *Pflugers Archiv: European Journal of Physiology*, **473**(7), 1009–1021.

Goversen, B., Van Der Heyden, M. A. G., Van Veen, T. A. B., & De Boer, T. P. (2018). The immature electrophysiological phenotype of iPSC-CMs still hampers in vitro drug screening: Special focus on IK1. *Pharmacology and Therapeutics*, **183**, 127–136.

Groenendaal, W., Ortega, F. A., Kherlopian, A. R., Zygmunt, A. C., Krogh-Madsen, T., & Christini, D. J. (2015). Cell-specific cardiac electrophysiology models. *PLoS Computational Biology*, **11**(4), 1–22.

Gutenkunst, R. N., Waterfall, J. J., Casey, F. P., Brown, K. S., Myers, C. R., & Sethna, J. P. (2007). Universally sloppy parameter sensitivities in systems biology models. *PLoS Computational Biology*, **3**(10), 1871–1878.

Han, L.u, Li, Y., Tchao, J., Kaplan, A. D., Lin, B.o, Li, Y., Mich-Basso, J., Lis, A., Hassan, N., London, B., Bett, G. C. L., Tobita, K., Rasmusson, R. L., & Yang, L. (2014). Study familial hypertrophic cardiomyopathy using patient-specific induced pluripotent stem cells. *Cardiovascular Research*, **104**(2), 258–269.

Hayes, H. B., Nicolini, A. M., Arrowood, C. A., Chvatal, S. A., Wolfson, D. W., Cho, H. C., Sullivan, D. D., Chal, J., Fermini, B., Clements, M., Ross, J. D., & Millard, D. C. (2019). Novel method for action potential measurements from intact cardiac monolayers with multiwell microelectrode array technology. *Scientific Reports*, **9**(1), 11893.

Herron, T. J., Da Rocha, A. M., Campbell, K. F., Ponce-Balbuena, D., Willis, B. C., Guerrero-Serna, G., Liu, Q., Klos, M., Musa, H., Zarzoso, M., Bizy, A., Furness, J., Anumonwo, J., Mironov, S., & Jalife, J. (2016). Extracellular matrix-mediated maturation of human pluripotent stem cell-derived cardiac monolayer structure and electrophysiological function. *Circulation: Arrhythmia and Electrophysiology*, **9**, 1–12.

Horváth, A., Lemoine, M. D., Löser, A., Mannhardt, I., Flenner, F., Uzun, A. U., Neuber, C., Breckwoldt, K., Hansen, A., Girdauskas, E., Reichenspurner, H., Willems, S., Jost, N., Wettwer, E., Eschenhagen, T., & Christ, T. (2018). Low resting membrane potential and low inward rectifier potassium currents are not inherent features of hiPSC-derived cardiomyocytes. *Stem Cell Reports*, **10**(3), 822–833.

Ismaili, D., Schulz, C., Horváth, A., Koivumäki, J. T., Mika, D., Hansen, A., Eschenhagen, T., & Christ, T. (2023). Human induced pluripotent stem cell-derived cardiomyocytes as an electrophysiological model: Opportunities and challenges-The Hamburg perspective. *Frontiers in Physiology*, **14**, 1132165.

Itzhaki, I., Maizels, L., Huber, I., Zwi-Dantsis, L., Caspi, O., Winterstern, A., Feldman, O., Gepstein, A., Arbel, G., Hammerman, H., Boulos, M., & Gepstein, L. (2011). Modelling the long QT syndrome with induced pluripotent stem cells. *Nature*, **471**(7337), 225–229.

Kernik, D. C., Morotti, S., Wu, H., Garg, P., Duff, H. J., Kurokawa, J., Jalife, J., Wu, J. C., Grandi, E., & Clancy, C. E. (2019). A computational model of induced pluripotent stem-cell derived cardiomyocytes incorporating experimental variability from multiple data sources. *The Journal of Physiology*, **597**(17), 4533–4564.

Lachaud, Q., Aziz, M. H. N., Burton, F. L., Macquaide, N., Myles, R. C., Simitev, R. D., & Smith, G. L. (2022). Electrophysiological heterogeneity in large populations of rabbit ventricular cardiomyocytes. *Cardiovascular Research*, **118**(15), 3112–3125.

Lee, J. H., Protze, S. I., Laksman, Z., Backx, P. H., & Keller, G. M. (2017). Human pluripotent stem cell-derived atrial and ventricular cardiomyocytes develop from distinct mesoderm populations. *Cell Stem Cell*, **21**(2), 179–194.e4.

Lei, C. L., Clerx, M., Whittaker, D. G., Gavaghan, D. J., de Boer, T. P., & Mirams, G. R. (2020). Accounting for variability in ion current recordings using a mathematical model of artefacts in voltage-clamp experiments. *Philosophical Transactions Series A, Mathematical, Physical, And Engineering Sciences*, **378**, 20190348.

Liang, P., Lan, F., Lee, A. S., Gong, T., Sanchez-Freire, V., Wang, Y., Diecke, S., Sallam, K., Knowles, J. W., Wang, P. J., Nguyen, P. K., Bers, D. M., Robbins, R. C., & Wu, J. C. (2013). Drug screening using a library of human induced pluripotent stem cell-derived cardiomyocytes reveals disease-specific patterns of cardiotoxicity. *Circulation*, **127**(16), 1677–1691.

López-Redondo, F., Kurokawa, J., Nomura, F., Kaneko, T., Hamada, T., Furukawa, T., & Yasuda, K. (2016). A distribution analysis of action potential parameters obtained from patch-clamped human stem cell-derived cardiomyocytes. *Journal of Pharmacological Sciences*, **131**(2), 141–145.

Ma, D., Wei, H., Lu, J., Huang, D., Liu, Z., Loh, L.i J., Islam, O., Liew, R., Shim, W., & Cook, S. A. (2015). Characterization of a novel KCNQ1 mutation for type 1 long QT syndrome and assessment of the therapeutic potential of a novel IKs activator using patient-specific induced pluripotent stem cell-derived cardiomyocytes. *Stem Cell Research and Therapy*, **6**(1), 39.

Ma, J., Guo, L., Fiene, S. J., Anson, B. D., Thomson, J. A., Kamp, T. J., Kolaja, K. L., Swanson, B. J., & January, C. T. (2011). High purity human-induced pluripotent stem cell-derived cardiomyocytes: Electrophysiological properties of action potentials and ionic currents. *American Journal of Physiology-Heart and Circulatory Physiology*, **301**(5), H2006–H2017.

Moretti, A., Bellin, M., Welling, A., Jung, C. B., Lam, J. T., Bott-Flügel, L., Dorn, T., Goedel, A., Höhnke, C., Hofmann, F., Seyfarth, M., Sinnecker, D., Schömig, A., & Laugwitz, K.-L. (2010). Patient-specific induced pluripotent stem-cell models for long-QT syndrome. *The New England Journal of Medicine*, **363**(15), 1397–1409.

Paci, M., Koivumäki, J. T., Lu, H. R., Gallacher, D. J., Passini, E., & Rodriguez, B. (2021). Comparison of the simulated response of three in silico human stem cell-derived cardiomyocytes models and in vitro data under 15 drug actions. *Frontiers in Pharmacology*, **12**, 604713.

Paci, M., Passini, E., Klimas, A., Severi, S., Hyttinen, J., Rodriguez, B., & Entcheva, E. (2020). All-optical electrophysiology refines populations of in silico human iPSC-CMs for drug evaluation. *Biophysical Journal*, **118**(10), 2596–2611.

Paci, M., Pölönen, R.-P., Cori, D., Penttinen, K., Aalto-Setälä, K., Severi, S., & Hyttinen, J. (2018). Automatic optimization of an in silico model of human iPSC derived cardiomyocytes recapitulating calcium handling abnormalities. *Frontiers in Physiology*, **9**, 709.

Prajapati, C., Ojala, M., Lappi, H., Aalto-Setälä, K., & Pekkanen-Mattila, M. (2021). Electrophysiological evaluation of human induced pluripotent stem cell-derived cardiomyocytes obtained by different methods. *Stem Cell Research*, **51**, 102176.

Quach, B., Krogh-Madsen, T., Entcheva, E., & Christini, D. J. (2018). Light-activated dynamic clamp using iPSC-derived cardiomyocytes. *Biophysical Journal*, **115**(11), 2206–2217.
Sala, L., Gnecchi, M., & Schwartz, P. J. (2019). Long QT syndrome modelling with cardiomyocytes derived from humaninduced pluripotent stem cells. *Arrhythmia & Electrophysiology Review*, **8**, 105–110.
Schmid, C., Abi-Gerges, N., Leitner, M., Zellner, D., & Rast, G. (2021). Ion channel expression and electrophysiology of singular human (Primary and induced pluripotent stem cell-derived) cardiomyocytes. *Cells*, **10**(12), 3370.
Seibertz, F., Rapedius, M., Fakuade, F. E., Tomsits, P., Liutkute, A., Cyganek, L., Becker, N., Majumder, R., Clauß, S., Fertig, N., & Voigt, N. (2022). A modern automated patch-clamp approach for high throughput electrophysiology recordings in native cardiomyocytes. *Communications Biology*, **5**(1), 969.
Van De Sande, D. V., Kopljar, I., Alaerts, M., Teisman, A., Gallacher, D. J., Loeys, B., Snyders, D. J., Leybaert, L., Lu, H. R., & Labro, A. J. (2021). The resting membrane potential of hSC-CM in a syncytium is more hyperpolarised than that of isolated cells. *Channels*, **15**(1), 239–252.
Verkerk, A., Veerman, C., Zegers, J., Mengarelli, I., Bezzina, C., & Wilders, R. (2017). Patch-clamp recording from human induced pluripotent stemcell-derived cardiomyocytes: Improving action potential characteristics throughdynamic clamp. *International Journal of Molecular Sciences*, **18**(9), 1873.
Verkerk, A. O., & Wilders, R. (2021). Dynamic clamp in electrophysiological studies on stem cell-derived cardiomyocytes-why and how? *Journal of Cardiovascular Pharmacology*, **77**(3), 267–279.
Workman, A. J., Pau, D., Redpath, C. J., Marshall, G. E., Russell, J. A., Kane, K. A., Norrie, J., & Rankin, A. C. (2006). Post-operative atrial fibrillation is influenced by beta-blocker therapy but not by pre-operative atrial cellular electrophysiology. *Journal of Cardiovascular Electrophysiology*, **17**(11), 1230–1238.
Zaniboni, M., Pollard, A. E., Yang, L., & Spitzer, K. W. (2000). Beat-to-beat repolarization variability in ventricular myocytes and its suppression by electrical coupling. *American Journal of Physiology-Heart and Circulatory Physiology*, **278**(3), H677–H687.
Zhang, J., Wilson, G. F., Soerens, A. G., Koonce, C. H., Yu, J., Palecek, S. P., Thomson, J. A., & Kamp, T. J. (2009). Functional cardiomyocytes derived from human induced pluripotent stem cells. *Circulation Research*, **104**(4), 30–41.

Additional information

Competing interests

The authors declare that they have no competing interests.

Author contributions

A.C. developed the figure and drafted the manuscript. A.C., T.K.M., and D.C. revised the manuscript. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding

This work was supported by the National Institutes of Health (NIH) National Heart, Lung, and Blood Institute (NHLBI) grants U01HL136297 (to D.C.) and F31HL154655 (to A.C.).

Keywords

digital twin, electrophysiology, experimental artifact, iPSC-CM, patch clamp, systems biology

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

Peer Review History