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# Using Light to Endow Stem-Cell-Derived Cardiomyocytes With Virtual $I_{K1}$ Conductances

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The availability of human stem cell-derived cardiomyocytes (hSC-CMs) has sparked a large interest in applying these cells in clinical and research settings. Pharmacological studies in particular may benefit from hSC-CMs because they provide an unlimited source of human cardiomyocytes. By comparison, human adult heart tissue is scarcely available, and when it is, the tissue is usually from explanted failing hearts or nonviable tissue obtained from autopsy. The benefit of a wide availability of hSC-CMs is, however, offset by their limited maturity in that their properties mostly resemble those of immature cardiomyocytes from early embryonic hearts. For instance, their cells are not quiescent but fire action potentials spontaneously, they have a cell structure that is not well differentiated, and they have limited sarcoplasmic reticulum function and a poorly developed network of transverse tubules. In the context of the human heart, hSC-CMs are most like sinoatrial node cells; however, for many studies, such as screening for drug-induced cardiac arrhythmias (e.g., torsades de pointes), ventricular cardiomyocytes may be more relevant and desirable.

The electrophysiological immaturity of hSC-CMs has been recognized as a problem in safety pharmacological studies (1–3). The spontaneous firing of action potentials and depolarized resting membrane potential are explained by a low expression of the potassium inward-rectifying $I_{K1}$ channels, which are essential for a hyperpolarized and stable resting membrane potential in muscle cells. As a consequence of the depolarized resting membrane potential, the other voltage-sensitive ion channels expressed by hSC-CMs will behave differently during the course of an action potential (4). For instance, $I_{Na}$ channels will be largely inactivated, resulting in a reduced contribution to the upstroke of the action potential. Also, $I_{Kr}$ channels, which are open only during the repolarization phase in adult cardiomyocytes, will remain open between action potentials. The altered ion channel states have consequences for the ability to predict pro- or antiarrhythmic properties that drugs may have on adult human hearts. Detecting effects of drugs blocking $I_{Kr}$ channels typically works well, but hSC-CMs have reduced sensitivity and predictive power for the effects of drugs blocking $I_{Ca,L}$, $I_{Na}$, or $I_{K1}$.

To overcome this problem, several groups have pursued strategies to increase the functional expression of $I_{K1}$ channels by employing virus-mediated overexpression of the *KCNJ2* gene encoding $I_{K1}$ channels (5,6) or employing dynamic clamping to inject real-time simulated $I_{K1}$ currents in patch-clamp experiments (7,8). Importantly, these studies have demonstrated that an increased functional expression of $I_{K1}$ indeed results in a more mature action potential with a normal, hyperpolarized resting membrane potential that is close to the reversal potential of $K^+$ ions. Normalization of action potential properties was accompanied by an improved sensitivity for drugs blocking $I_{Na}$ and $I_{Ca,L}$, which greatly improves the relevance of hSC-CMs for future large-scale drug testing.

Thus, studies that involve recording action potentials using patch electrodes can be enhanced by a dynamic clamp-based addition of virtual $I_{K1}$ currents. Unfortunately, this approach is rather laborious and has a low throughput, although the combination of dynamic clamping and automated patch-clamp devices can increase throughput (9). A potential approach to further increase throughput would be to employ an all-optical electrophysiological method in which membrane potential is recorded using a voltage-sensitive optogenetic sensor or voltage-sensitive dye and $I_{K1}$ conductance is shaped in a light-sensitive manner. Such an approach would allow the tuning of $I_{K1}$ in single cells (which is essential because each cell is different) and circumvent the need to physically connect an electrode to each individual cell, thereby enabling an increased throughput by several orders of magnitude.

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In their current contribution to this journal, Quach et al. (10) have advanced the field by making a big step toward this goal. Given that there is no available method to directly control the function of $I_{K1}$ channels using light, Quach et al. (10) have creatively used dynamic clamping to confer $I_{K1}$-like kinetics to hyperpolarizing, outward currents generated by the light-activated ArchT, opsin. By defining a suitable transfer function between membrane potential and light intensity, it was possible to effectively inject $I_{K1}$ into human-induced pluripotent stem cell-derived cardiomyocytes and obtain more mature action potentials, which approached those recorded from the same cells using traditional current injection-based dynamic clamping. This study demonstrates that employing an optical dynamic clamp (ODC) is feasible and may lead to the development of true all-optical electrophysiology combined with a light-based dynamic clamp. There are several obstacles that have to be cleared, one being that the current ODC method relies on a patch-clamp electrode to record membrane potential. Replacing this with an optical fluorescence signal is challenging because signals from the current generation of voltage-sensitive dyes or sensors have a much lower signal/noise ratio, complicating reliable dynamic clamping. Also, dynamic clamping requires a fast update frequency, which is complicated with a fluorescence signal because faster acquisition will reduce the photon count per sample. Implementation of the light-based dynamic clamp will also require the development of reliable means for calibration of voltage sensor/dye signals because these do not give an absolute membrane potential but, for now, give relative measures of voltage. Despite the challenges remaining, a significant step has been made toward reaching the goal of high-throughput drug testing using human cardiomyocytes with enhanced maturity. Whether an ODC can enhance predictions of the effects of drugs blocking $I_{Na}$ or $I_{Ca,L}$ remains to be determined, but the outlook is promising.

## REFERENCES


1. Blinova, K., J. Stohlman, …, D. G. Strauss. 2017. Comprehensive translational assessment of human-induced pluripotent stem cell derived cardiomyocytes for evaluating drug-induced arrhythmias. *Toxicol. Sci.* 155:234–247.
2. Ando, H., T. Yoshinaga, …, Y. Sekino. 2017. A new paradigm for drug-induced torsadogenic risk assessment using human iPS cell-derived cardiomyocytes. *J. Pharmacol. Toxicol. Methods.* 84:111–127.
3. Goversen, B., M. A. G. van der Heyden, …, T. P. de Boer. 2017. The immature electrophysiological phenotype of iPSC-CMs still hampers in vitro drug screening: special focus on IK1. *Pharmacol. Ther.* 183:127–136.
4. Jonsson, M. K., M. A. Vos, …, T. A. van Veen. 2012. Application of human stem cell-derived cardiomyocytes in safety pharmacology requires caution beyond hERG. *J. Mol. Cell. Cardiol.* 52:998–1008.
5. Vaidyanathan, R., Y. S. Markandeya, …, L. L. Eckhardt. 2016. IK1-enhanced human-induced pluripotent stem cell-derived cardiomyocytes: an improved cardiomyocyte model to investigate inherited arrhythmia syndromes. *Am. J. Physiol. Heart Circ. Physiol.* 310:H1611–H1621.
6. Li, M., Y. Kanda, …, J. Kurokawa. 2017. Overexpression of KCNJ2 in induced pluripotent stem cell-derived cardiomyocytes for the assessment of QT-prolonging drugs. *J. Pharmacol. Sci.* 134:75–85.
7. Bett, G. C., A. D. Kaplan, …, R. L. Rasmusson. 2013. Electronic "expression" of the inward rectifier in cardiocytes derived from human-induced pluripotent stem cells. *Heart Rhythm.* 10:1903–1910.
8. Meijer van Putten, R. M., I. Mengarelli, …, R. Wilders. 2015. Ion channelopathies in human induced pluripotent stem cell derived cardiomyocytes: a dynamic clamp study with virtual IK1. *Front. Physiol.* 6:7.
9. Goversen, B., N. Becker, …, T. P. de Boer. 2018. A hybrid model for safety pharmacology on an automated patch clamp platform: using dynamic clamp to join iPSC-derived cardiomyocytes and simulations of $I_{k1}$ ion channels in real-time. *Front. Physiol.* 8:1094.
10. Quach, B., T. Krogh-Madsen, …, D. J. Christini. 2018. Light-activated dynamic clamp using iPSC-derived cardiomyocytes. *Biophys. J.* 115:2206–2217.