

# Human Induced Pluripotent Stem Cell Derived Cardiomyocytes for Assessing Drug-induced Cardiac Arrhythmias

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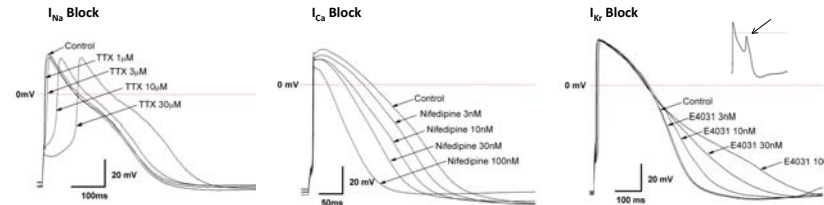
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## Abstract

The potential for drug-induced cardiac arrhythmias is a vital component of the toxicological and safety pharmacological profile of new chemical entities (NCEs). Cardiomyocytes contain multiple ion channels and ion transporters and cardiac electrical activity arises from the temporal and spatial activity of these proteins. Commonly employed methods fail to recapitulate the human condition as the predominant systems typically express a single channel without ancillary proteins and may therefore miss exacerbating or compensatory protein interactions, while animal models may ultimately miss species-specific effects. **iCell™ Cardiomyocytes** are derived from human induced pluripotent stem cells (hiPSCs), fully functional, surmount many of the hurdles associated with traditional models, exhibit provide relevant drug induced effects, and provide an excellent system for assessing the arrhythmogenic potential of NCEs

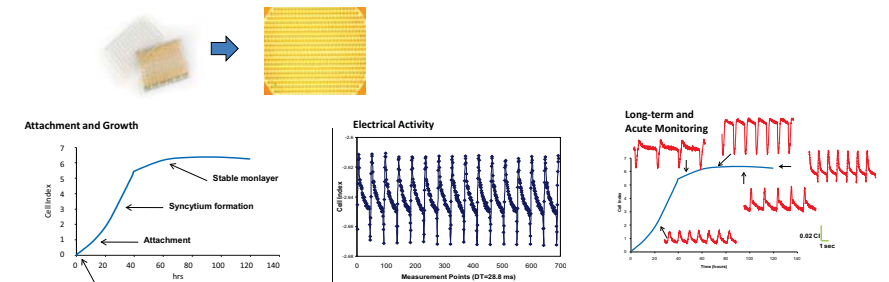
## iCell Cardiomyocytes exhibit the expected effects on action potential parameters in response to ion channel block



| % of control      | Dose  | Peak      | MDP       | APD10      | APD50      | APD90       | dV/dt      |
|-------------------|-------|-----------|-----------|------------|------------|-------------|------------|
| TTX<br>N=5        | 1µM   | 103.8±3.0 | 100.3±0.9 | 107.1±2.5  | 103.1±1.9  | 101.7±2.0   | 76.7±7.4   |
|                   | 3µM   | 100.0±1.3 | 99.5±0.8  | 105.7±4.3  | 100.6±2.5  | 98.8±0.8    | 41.2±11.2* |
|                   | 10µM  | 99.0±2.7  | 97.6±1.1  | 108.8±6.6  | 98.3±4.3   | 96.4±3.7    | 16.7±1.8*  |
|                   | 30µM  | 99.1±3.6  | 96.2±2.1  | 112.4±6.3  | 102.0±3.4  | 100.2±3.1   | 16.8±2.0*  |
|                   | 100µM | 99.0±1.0  | 99.8±0.6  | 94.8±3.4   | 95.5±1.8   | 98.7±2.0    | 98.9±4.4   |
| E4031<br>N=5      | 10nM  | 100.5±1.2 | 98.3±0.8  | 92.2±2.8   | 100.5±1.6  | 112.8±2.8   | 98.2±5.9   |
|                   | 30nM  | 99.3±1.0  | 97.4±1.2  | 90.1±3.0   | 109.1±3.7* | 140.3±7.6*  | 90.1±7.6   |
|                   | 100nM | 101.1±1.4 | 94.2±2.6  | 83.0±10.2  | 113.4±3.9* | 170.4±13.6* | 69.4±17.1  |
|                   | 3µM   | 89.5±4.9  | 99.5±0.4  | 83.3±7.1*  | 84.6±2.4*  | 89.4±1.0*   | 83.9±14.5  |
|                   | 10µM  | 82.2±9.0  | 98.7±0.8  | 65.0±12.3* | 70.3±6.1*  | 78.4±4.4*   | 91.3±4.4   |
| Nifedipine<br>N=5 | 30nM  | 87.2±6.9  | 97.3±1.8  | 60.9±7.6*  | 65.7±3.8*  | 74.0±2.3*   | 84.8±11.2  |
|                   | 100nM | 73.6±12.2 | 96.6±2.0  | 31.7±11.1* | 45.4±4.5*  | 58.2±5.4*   | 87.7±8.6   |

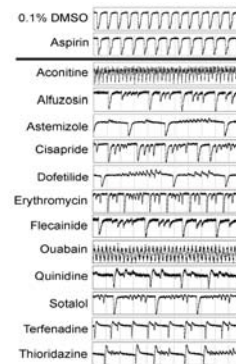
**Effects of ion channel block on action potential parameters.** Conventional single cell current clamp perforated patch techniques were used to characterize the effects of specific ion channel block on the action potential parameters. Experiments were conducted at 37°C and cells were paced at 1Hz. Sodium channel block by tetrodotoxin (TTX) slowed the upstroke velocity. hERG channel block by E4031 caused a prolongation of the action potential and could elicit early after-depolarizations (EADs; inset). Calcium channel block by nifedipine caused a shortening of the action potential.

## iCell Cardiomyocytes show predictive drug-induced pro-arrhythmic effects under higher throughput impedance measurements



**Label-free impedance measurements.** The xCelligence RTCA is a 96-well based system that uses impedance as a surrogate measurement of cell coverage and electrical activity. The cell index increases as cells attach and from a syncytial monolayer (left). Syncytial contractions occurring as a result of rhythmic electrical activity are recorded as oscillations in cell index (middle).

## Arrhythmia Screening in 96-wells

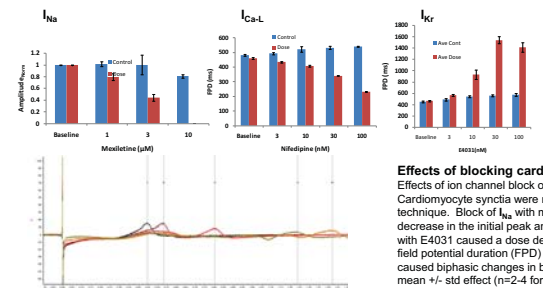


## Exhibit a predictive advantage over current models

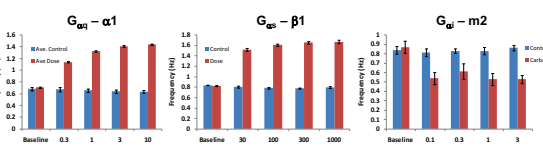
| Drug         | PPS  | hERG | QT  | Clinical Arrhythmia |
|--------------|------|------|-----|---------------------|
| Quabain      | 5667 | (-)  | (-) | (*)                 |
| Aconitine    | 2567 | (-)  | (-) | (*)                 |
| Quinidine    | 2158 | (*)  | (*) | (*)                 |
| Dofetilide   | 2000 | (*)  | (*) | (*)                 |
| Flecainide   | 1931 | (*)  | (*) | (*)                 |
| Erythromycin | 1135 | (*)  | (*) | (*)                 |
| Terfenadine  | 1000 | (*)  | (*) | (*)                 |
| Thioridazine | 594  | (*)  | (*) | (*)                 |
| RO5657       | 555  | (*)  | (*) | (*)                 |
| Sotalol      | 491  | (*)  | (*) | (*)                 |
| Cisapride    | 429  | (*)  | (*) | (*)                 |
| E-4031       | 433  | (*)  | (*) | (*)                 |
| Astemizole   | 262  | (*)  | (*) | (*)                 |
| Ranolazine   | 60   | (*)  | (*) | (-)                 |
| Alifuzosin   | 56   | (-)  | (-) | (-)                 |
| Fluoxetine   | NA   | (*)  | (*) | (-)                 |
| Moxifloxacin | NA   | (*)  | (*) | (*)                 |
| Amiodarone   | NA   | (*)  | (*) | (*)                 |
| Verapamil    | NA   | (*)  | (*) | (-)                 |
| Captopril    | NA   | (-)  | (-) | (-)                 |
| Nifedipine   | NA   | (-)  | (-) | (-)                 |
| Amoxicillin  | NA   | (-)  | (-) | (-)                 |
| Rofecoxib    | NA   | (-)  | (-) | (-)                 |
| Aspirin      | NA   | (-)  | (-) | (-)                 |

**Predicting Proarrhythmia with iCell Cardiomyocytes.** The left hand figures illustrate normal and arrhythmic beating during exposure to innocuous and cardioactive compounds, respectively. The table on the right compares the predicted proarrhythmia score (calculated as a function of ectopic beats in relation to the Cmax of the compound) to traditional in-vitro hERG and QT assays as well as in-vivo data. iCell cardiomyocytes faithfully detected positive and negative drugs, as well as pro-arrhythmic drugs that were not detected by the in-vitro assays (false negatives – purple box) as well as non-arrhythmic compounds deemed hazardous by the in-vitro assays (false positives green box).

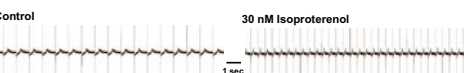
## iCell Cardiomyocytes exhibit the expected effects on extracellular field potentials parameters in response to ion channel block



**Effects of blocking cardiac ionic currents.** Effects of ion channel block on autonomously beating iCell Cardiomyocyte syncytia were measured with the multielectrode array (MEA) technique. Block of  $I_{Na}$  with mexiletine caused a dose dependent decrease in the initial peak amplitude. Block of  $I_{Ca-L}$  with nifedipine or  $I_{Kr}$  with E4031 caused a dose dependent shortening or prolongation of the field potential duration (FPD) respectively, while block of  $I_{K}$  with ZD2288 caused biphasic changes in beat frequency. The bar graph illustrates the mean +/- std effect (n=2-4 for each group). The tracing illustrates the effects of E4031 on FPD duration where cursors (1-5) mark the FPD for control, 3, 10, 30, and 100 nM E4031, respectively.

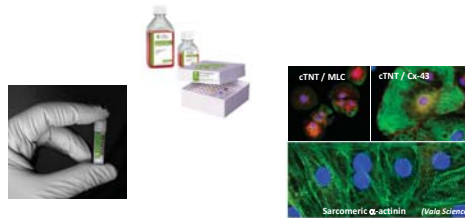


**Effects of G-protein coupled receptor stimulation.** Stimulation of  $G_{\alpha-1}$  with phenylephrine or  $G_{\alpha-1}$  with isoproterenol caused an increase in autonomous beat frequency, while stimulation of  $G_{\alpha-2}$  caused a decrease in autonomous beat frequency. The bar graphs illustrate the mean +/- std (n = 2-4 for each group). The tracings show representative recordings illustrating the effects of 30 nM Isoproterenol on beat frequency.

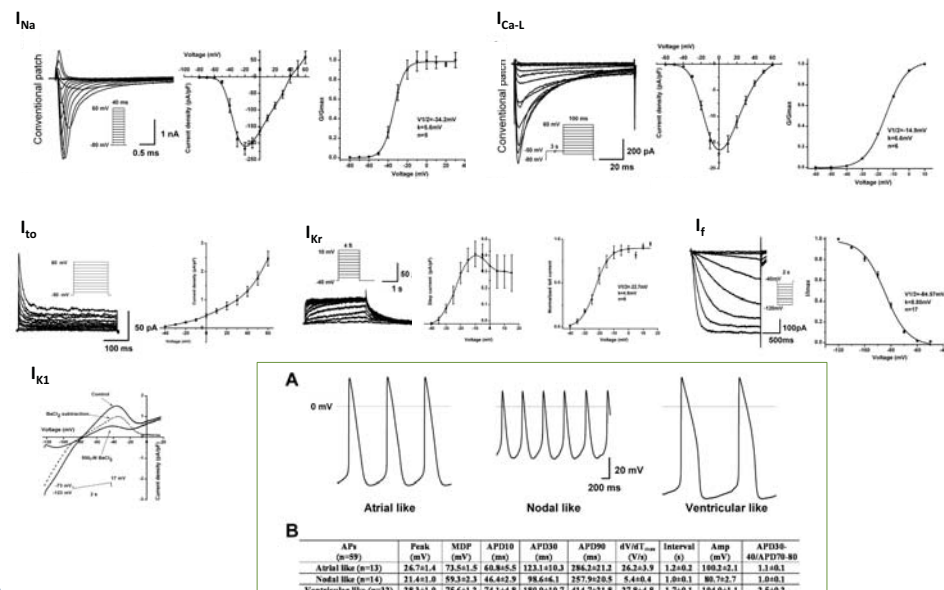


## iCell™ Cardiomyocytes

- Human iPS cell-derived
- 99% pure, cryopreserved, ready to use.
- Available in virtually unlimited quantities
- Full product solution; cells, media, protocols
- Express all function cardiac markers including critical ion channels
- Demonstrate normal human cardiac biology and electrophysiology



## iCell Cardiomyocytes express typical cardiac ion channels and exhibit cardiac like action potentials.



**Cardiac ion channel and action potential characterization.** Conventional single cell voltage and current clamp techniques were used to characterize multiple cardiac ionic currents and resulting action potentials contained within iCell Cardiomyocytes.

## Summary

**iCell Cardiomyocytes are a human-based cardiomyocyte test system that:**

1. Show the relevant cardiac electrophysiology
2. Exhibit expected responses in traditional pro-arrhythmia assays
3. Demonstrate an increased proarrhythmia predictive power over traditional systems when assayed in a higher throughput surrogate system