Human iPSC-derived hepatocytes and cardiomyocytes for drug toxicity testing

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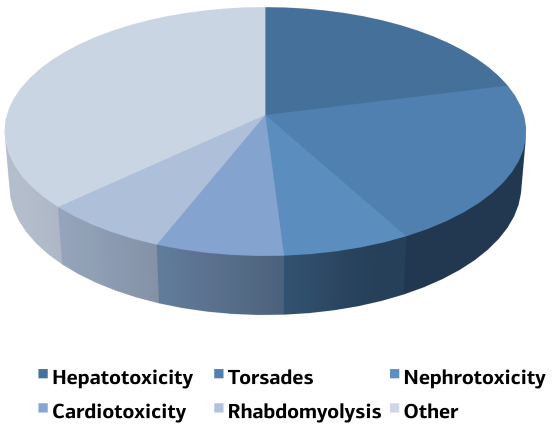
INTRODUCTION

Pharmaceutical candidate compounds require an extended period of time and high development costs before reaching the market. However, many candidate compounds are eliminated in the development process. Candidate compound attrition is frequently based on hepatotoxicity and cardiotoxicity (torsades de pointes). Although human primary cells are widely used for drug toxicity testing, they are subject to high lot-to-lot variability. Moreover, it is difficult to perform long-term tests with the same donor when using human primary cells due to their limited supply.



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Human-derived iPS cells are pluripotent stem cells with the capacity for infinite proliferation and differentiation into various cells including hepatocytes and cardiomyocytes. iPS cells enable unlimited production of differentiated cells from a single donor, possessing the same genetic background. Donor-derived iPS cells can be established from a wide variety of individuals, allowing access to differentiated cells, such as hepatocytes and cardiomyocytes, from highly varied genetic backgrounds.

MATERIALS & METHODS

ReproHepato Type I™ Kit (1 kit for 96-well plate) (ReproCELL Cat. No. RCESDH001)

- Cells, 1 vial (8.25 million cells/vial)
- Thawing Medium, 1 bottle
- Maintenance Medium, 1 bottle
- Assay Medium, 1 bottle
- Supplements

Hepatotoxicity Assay

- Acetaminophen (Sigma #A7085)
- Amiodarone hydrochloride (Sigma #A8423)
- Cyclophosphamide monohydrate (Sigma #C0768)
- Flutamide (Sigma #F9397)
- CellTiter-Glo™ Cell Viability Assay (Promega #G7571)
- Cytotoxicity Detection Kit^{PLUS} (LDH) (Roche #4744936)
- ARVOX3 (PerkinElmer Japan)

High Content Analysis

- Cell Insight NXT™ (ThermoFisher)
- Drug Induced Liver Injury (DILI) Cartilage (ThermoFisher)

ReproCardio2™ Kit (1 kit for 96-well plate) (ReproCELL Cat. No. RCESD008)

- Cells 3 vials (3.3×10^5 single cells/vial)
- Maintenance culture medium (80mL) x 2
- Coating solution (30mL) x 1
- Low attachment plate for aggregated cells
- Attachment plate for thin-layer / single cell

Immunocytochemistry

Primary antibodies:

- Anti-Conexin 43, MYH6/7(Sigma)
- Anti-MLC-2A/2V (Synaptic systems)
- Anti-Troponin (AbD)
- Anti-Vimentin (Millipore)

Secondary antibody:

- Alexa Fluor™ 488 (Life Technologies)

Electrophysiological Assay

- Equipment: aMED, AXION, MCS
- Coating: Fibronectin

Characterization of ReproHepato

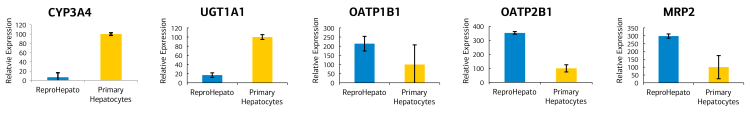


FIGURE 1. ReproHepato expresses standard hepatocyte markers for drug metabolizing enzymes and drug transporters. The mRNA levels of various hepatocyte specific markers measured by qPCR relative to primary hepatocytes (set to 100).

Drug Toxicity Testing using ReproHepato ATP and LDH Assay

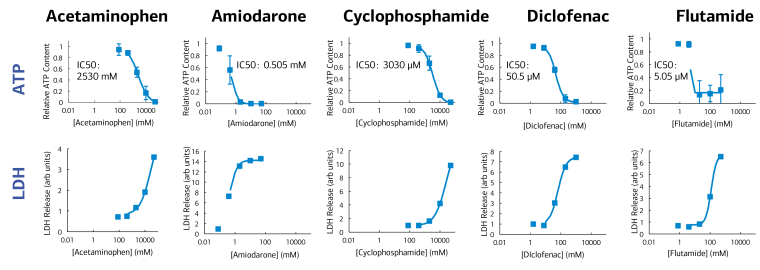
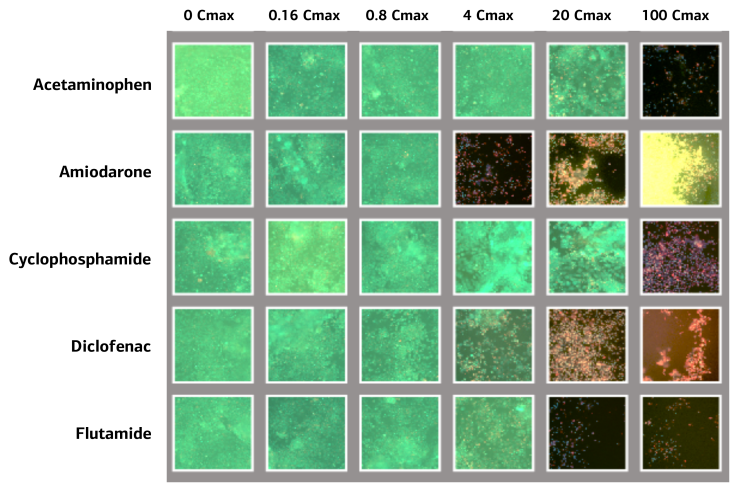


FIGURE 2. ReproHepato cells show toxic responses to standard xenobiotics. After exposure of ReproHepato to hepatotoxins for 48 hours, ATP content and LDH release were measured, showing concentration-dependent reduction of cell viability.

High Content Analysis After Drug Exposure



PARAMETER	LOCALIZATION	COLOR
Cell number	Nuclear	Blue
DNA content	Nuclear	Blue
Glutathione Content (GSH)	Cytoplasm	Green
Reactive Oxygen Species (ROS)	Whole cell	Yellow
Mitochondrial membrane potential (MMP)	Cytoplasmic	Red

FIGURE 3. ReproHepato cells facilitate HCS analysis of toxicity. ReproHepato cells were treated for 48 hours with five known hepatotoxins, and analyzed using a Cell Insight NXT Imager. These results show a variety of potential toxic mechanisms are active in ReproHepato cells.

CONCLUSIONS

- Human iPS-derived hepatocytes (ReproHepato) and cardiomyocytes (ReproCardio 2) express normal markers matching their particular cell types.
- ReproHepato cells show similar response to primary hepatocytes in standard *in vitro* toxicity assays.
- ReproHepato cells facilitate HCS analysis of hepatotoxicity.
- ReproCardio 2 cells enable MEA assays for in vitro prediction of cardiotoxicity.

Characterization of ReproCardio 2

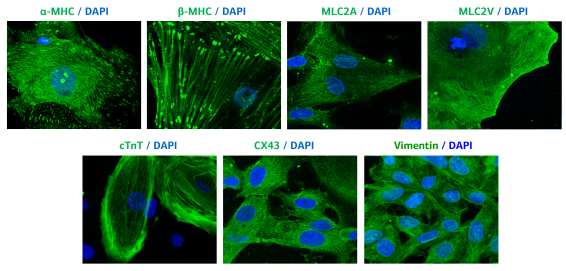
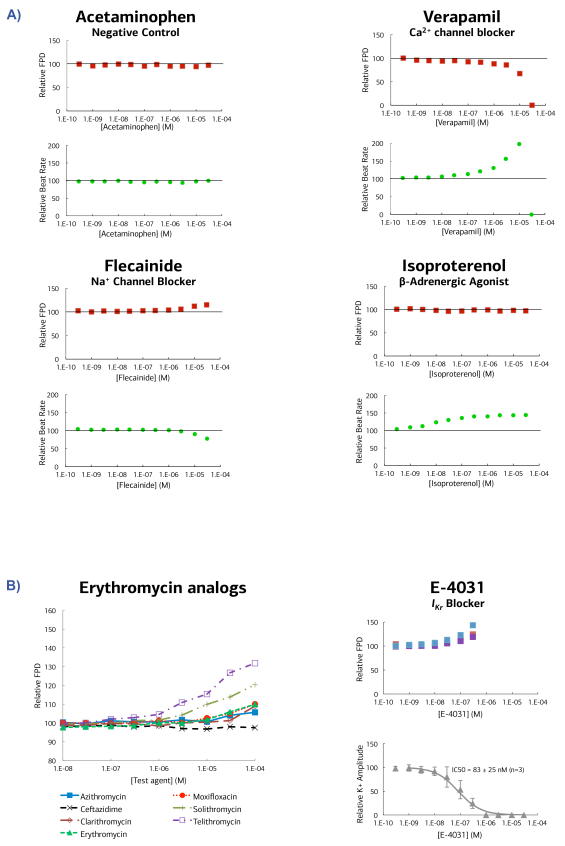


FIGURE 4. ReproCardio 2 cells express standard cardiomyocyte markers. α-MHC and β-MHC are the representative cardiac markers during differentiation from iPS cells and expressed in heart. MLC-2A and MLC-2V are myosin specific markers for the atrium of the mammalian heart and for the ventricle of the mammalian heart, respectively. Cardiac troponin T (cTnT) is a specific marker in human heart and a thin filament protein which takes part in muscle contraction. Cx43 is a connexin gap junction protein.

MEA Assay using ReproCardio 2



Test Agent	1 nM	3 nM	10 nM	30 nM	100 nM	300 nM	1 µM	3 µM	10 µM	30 µM	100 µM
Azithromycin	100	102	100	100	101	101	101	102	101	104	106
Ceftazidime	101	100	98	98	99	98	99	97	97	98	98
Clarithromycin	100	99	99	99	100	100	98	100	100	101	109
Erythromycin	99	100	98	98	98	99	100	100	102	106	110
Moxifloxacin	102	101	100	100	100	100	101	100	103	106	110
Solithromycin	100	99	99	100	100	101	102	104	110	114	120
Telithromycin	101	100	100	100	102	103	105	111	115	127	132

FIGURE 5. ReproCardio 2 cells are sensitive to electrophysiological effects of drugs by MEA. Thin-layer ReproCardio 2 cells were exposed to various concentrations of drugs from 300 pM to 30 µM. The cells were analyzed by MEA assay using an MCS system, with 2 minutes of drug exposure and 2 minutes of recording. **A)** Field Potential Duration (FPD) and beat rate changes were measured for potential cardiotoxins. **B)** Three different lots of ReproCardio 2 were exposed to various concentrations of E4031. The three different batches exhibit similar Field Potential Duration, indicating low lot-to-lot variability.

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