Human iPSC-derived hepatocytes ReproHepato™ for CYP assay & drug toxicity testing

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Abstract & Objective Mass production of hepatocytes with high stability and low lot-to-lot variation

Pharmaceutical candidate compounds require extended period of time and large amount of development costs before reaching the market. However, for various reasons, most of the candidates will be canceled in their development process. One major reason is hepatotoxicity. Recently, in the beginning of the drug screening process, simple and fast evaluation by so-called "Cell-based assay" has been given great importance in terms of safety and cost reduction. Although human primary hepatocytes have been widely used for hepatotoxicity, the following problems still remain: lot-to-lot variation, commercial availability and unstable supply. Moreover, there is a great difficulty in executing long-term tests using hepatocytes from the identical donor.
Human-derived iPS cells are pluripotent stem cells with the ability of infinite proliferation and differentiation into various cells including hepatocytes. Therefore, iPS cells enable unlimited production of hepatocytes possessing the same genetic back ground. Furthermore, it is possible to produce various donor-derived hepatocytes since human-derived iPS cells can be establish from varied race, sex and genetic back ground.

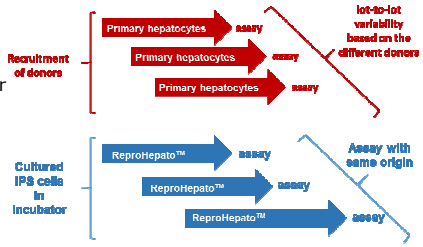


Fig. 1. Stable supply of human iPS cell-derived hepatocyte applicable for toxicity screening
Upper: Primary hepatocytes. Lower: human iPS cell-derived hepatocyte (ReproHepato™)
There is a limit in cell supply from the identical donor and lot-to-lot variation between each donors. On the other hand, human-derived iPS cells from the identical donor are capable of infinite proliferation and are expected to overcome the problems mentioned above.

Materials & Methods

ReproHepato type I™ kit (1 kit for 1 plate) (ReproCELL #RCESDH001)
- Cells 1 vial (8.25 million cells/vial)
- Thawing Medium 1 bottle
- Maintenance medium 1 bottle
- Assay Medium 1 bottle
- Supplements

CYP3A4 induction assay
- P450-Glo™ CYP3A4 Assay (Luciferin-PFBE) Cell-Based/Biochemical Assay, V8901 (Promega Corporation, Madison, WI, USA)
- Functional Drug Screening System FDSS/μCELL (Hamamatsu Photonics K.K., Shizuoka, Japan)
- Greiner tissue culture treated 96 well plates (Greiner , Frickenhausen, German)

Hepatotoxicity assay
- Acetaminophen (Sigma #A7085)
- Amiodarone hydrochloride (Sigma #A8423)
- Cyclophosphamide monohydrate (Sigma #C0768)
- Diclofenac sodium salts (Sigma #D6899)
- Flutamide (Sigma #F9397)
- CellTiter-Glo™ Luminescent Cell Viability Assay (Promega #G7571)
- Cytotoxicity Detection Kit^{PLUS} (LDH) (Roche #4744936)
- ARVOX3 (PerkinElmer Japan)

High Contents Analysis
- Cell Insight NXT (Thermo Fisher)
- Drug Induced Liver Injury (DILI) Cartilage (Thermo Fisher)

Results : Characterization

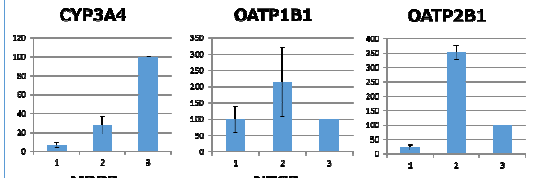
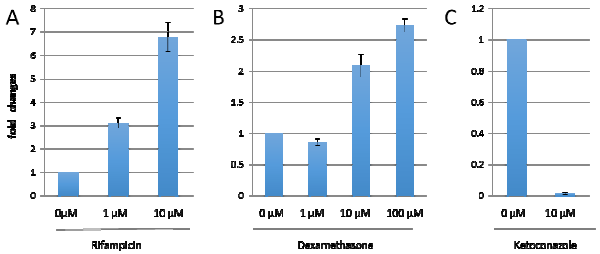


Fig. 2. mRNA expression in ReproHepato™.
The Expression levels of various hepatocyte specific markers were comparative to those of Primary Hepatocytes.

1: Frozen ReproHepato™ 3lots
2: Fresh ReproHepato™ 3lots
3: Primary Hepatocytes 1lot
On the y-axis, the level of the Primary Hepatocytes was taken as 100%.

Results : CYP Assay

Fig. 3. The changes of CYP3A4 activity of ReproHepato™ in a dose-dependent manner, by rifampicin, dexamethasone, and ketoconazole as in the case of primary hepatocytes.
CYP3A4 activity of ReproHepato™ is induced by rifampicin (A) and dexamethasone (B), and inhibited by ketoconazole (C), respectively, in a dose-dependent manner. ReproHepato™ show similar kinetics with primary hepatocytes in CYP3A4 assay.



Results : Drug Toxicity Testing

ATP and LDH Assay Using ReproHepato™

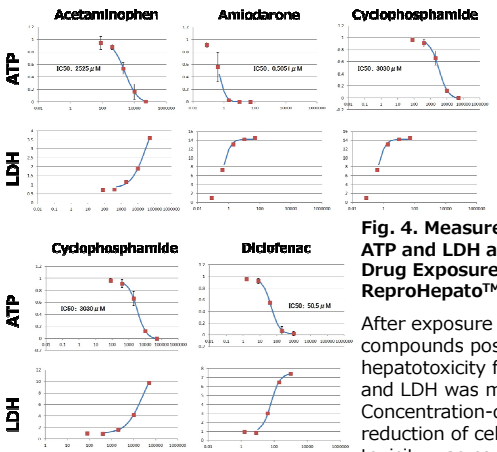
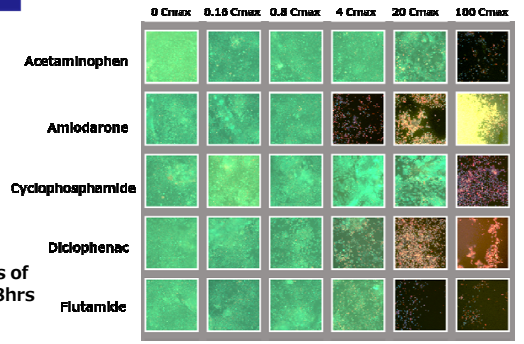


Fig. 4. Measurements of ATP and LDH after 48hrs Drug Exposure to ReproHepato™

After exposure of five compounds possessing hepatotoxicity for 48 hours, ATP and LDH was measured. Concentration-dependent reduction of cell viability and toxicity was confirmed.

High Contents Analysis After Drug Exposure



parameter	position	color
cell number	nuclear	blue
DNA content	nuclear	blue
glutathione (GSH)	cyteplasm	green
reactive oxygen species (ROS)	whole cells	yellow
mitochondrial membrane potential (MMP)	nuclear, cytoplasm	red

Fig. 5. High content analysis by Cell insight NXT
After exposure of five compounds possessing hepatotoxicity for 48 hours, photography and measurements of the dyed and agent-treated cells with Drug Induced Liver Injury (DILI) cartilage were done using cell imaging analyzer: Cell insight NXT. Concentration of the exposed compounds has been changed by 5-fold dilution to prepare six type of concentration. Here, 5 information such as mitochondrial membrane potential was extracted.

Conclusion

- In this study, we developed human iPS cells-derived hepatocytes (ReproHepato™), which can be used for CYP3A4 induction assays.
- Concentration-dependent toxicity has been confirmed by measurements of ATP and LDH.
- By high content analysis, the following were simultaneously measured: cell number, intranuclear DNA, reduction in glutathione level, active oxygen and mitochondrial membrane potential.