Stem Cell-Derived Hepatocyte-Like Cells: An Alternative for High Throughput Toxicity Screening

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Abstract

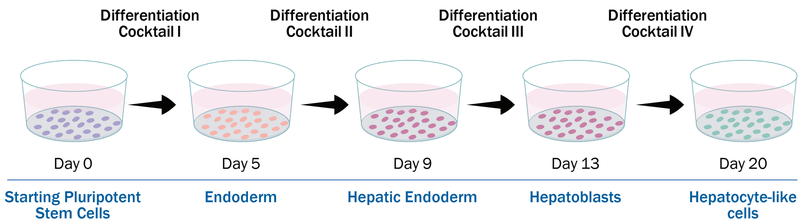
Secondary liver toxicity is a leading cause of commercial pharmaceutical drug recalls and compound failures during drug development. Primary hepatocytes, the most common *in vitro* model for drug-induced liver toxicity testing, are not ideal for high throughput screening due to their limited availability, difficulty to culture, and functional instability *in vitro*. Hepatocyte-like cells, derived from human pluripotent stem cells, are emerging as a stable and renewable model for drug-induced liver toxicity testing. In this study, we hypothesize that hepatocyte-like cells derived from induced pluripotent stem cells using the StemXVivo® Hepatocyte Differentiation Kit can be used for high throughput toxicity screens. We have previously demonstrated that the StemXVivo® Hepatocyte Differentiation Kit efficiently and consistently generates functional hepatocyte-like cells. To provide further functional validity of these cells we first show that Cyp3A4, an essential hepatic enzyme often analyzed in drug toxicity studies, can be induced in kit-derived hepatocytes. We then used high content imaging to quantitate cell viability against a panel of known hepatotoxic molecules. Our data indicate that the StemXVivo® Hepatocyte Differentiation Kit provides a reliable and renewable source of hepatocyte-like cells that can be utilized for high throughput toxicology and drug discovery.

StemXVivo® Hepatocyte Differentiation Kit

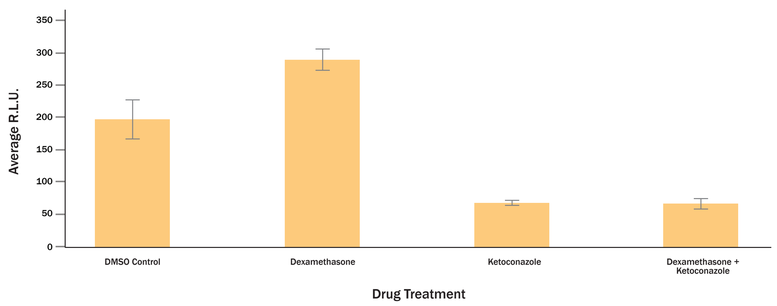
Kit Contents

- Hepatocyte Differentiation Base Media Supplement I
- Hepatocyte Differentiation Base Media Supplement II
- Hepatocyte Differentiation Cocktail I
- Hepatocyte Differentiation Cocktail II
- Hepatocyte Differentiation Cocktail III
- Hepatocyte Differentiation Cocktail IV
- Anti-HNF-4α Antibody

Differentiation Protocol

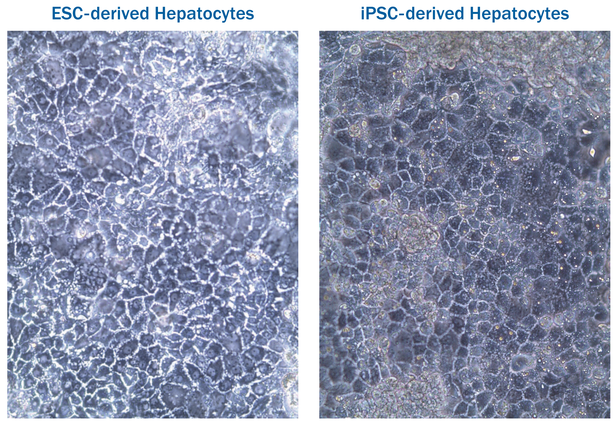


Functional Cyp3A4 Activity in Pluripotent Stem Cell-derived Hepatocytes

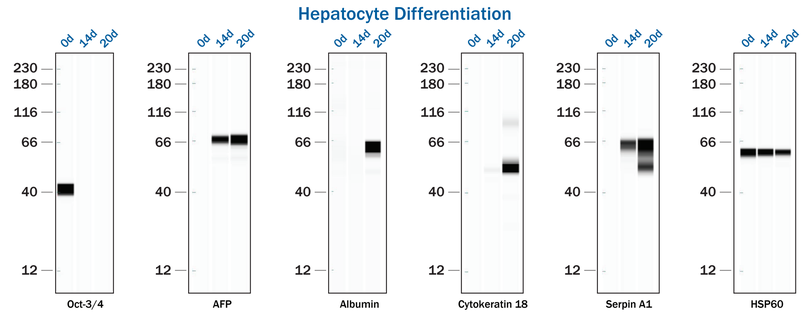


Induction and Activity of Cyp3A4 Expression in Hepatocytes Derived from Human Embryonic Stem Cells. Hepatocyte-like cells differentiated from BG01V human embryonic stem cells using the differentiation kit were treated for 48 hours with 50 µM Dexamethasone (Catalog # 1126) and/or 20 µM Ketoconazole (Catalog # 1103) to induce and inhibit Cyp3A4 expression, respectively. Dexamethasone treatment resulted in an increase in Cyp3A4 activity compared to untreated DMSO alone controls. Ketoconazole treatment inhibited Cyp3A4 activity in untreated and Dexamethasone-treated samples. Cyp3A4 activity was measured using the P450-Glo CYP3A4 cell-based assay (Promega).

Pluripotent Stem Cell-derived Hepatocyte-like Cells Display Characteristic Hepatocyte Morphology and Cell-specific Protein Expression

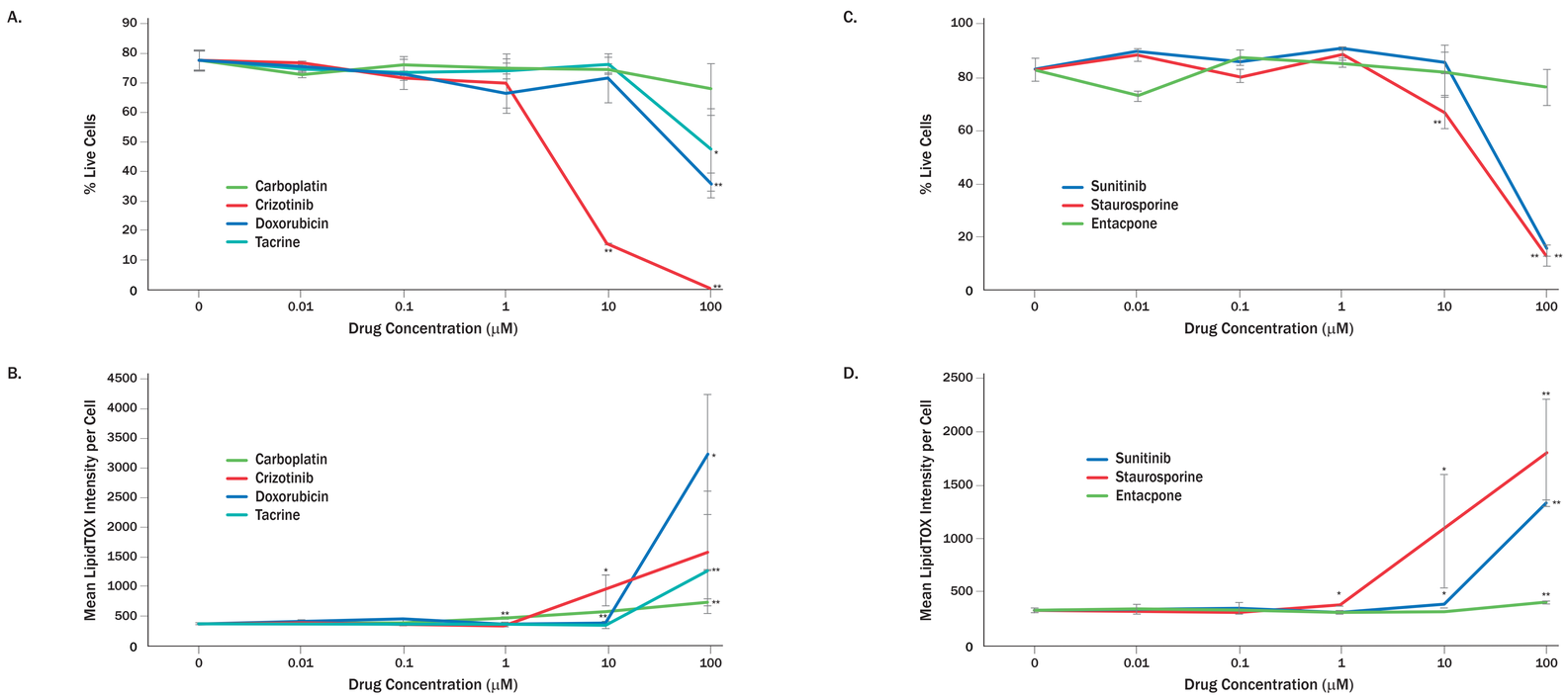


Stem Cell-derived Hepatocyte-like Cells have Characteristic Hepatocyte Morphology. BG01V human embryonic stem cells and iPSK3 induced pluripotent stem cells were differentiated into hepatocyte-like cells using the differentiation kit. Brightfield images of BG01V-derived and iPSK3-derived hepatocytes show the characteristic cobblestone morphology of hepatocytes grown *in vitro*.



Hepatocyte-specific Protein Expression in Pluripotent Stem Cell-derived Hepatocyte-like Cells. iBJ6 human induced pluripotent stem cells were differentiated into hepatocyte-like cells using the differentiation kit. Cells at day 0, 14, and 20 of differentiation were collected, lysed, and analyzed by Simple Western™ for hepatocyte-specific protein expression. Expression of the stem cell marker, Oct-3/4 (Catalog # AF1759), was observed in undifferentiated iBJ6 cells, but not in cells differentiating into hepatocyte-like cells. Hepatocyte-like cells showed increasing expression of hepatocyte-specific proteins, alpha-Fetoprotein (AFP; Catalog # MAB1368), Serum Albumin (Catalog # AF3329),Cytokeratin 18 (Catalog # MAB7619), and Serpin A1(Catalog # AF1268). HSP60 (Catalog # MAB1800) was used as a housekeeping protein.

Pluripotent Stem Cell-derived Hepatocyte-like Cells as an *in vitro* Liver Toxicity Model



Hepatotoxicity to Small Molecules Assessed Using High Content Imaging. iPSK3 (**A**,**B**) and iBJ6 (**C**,**D**) human pluripotent stem cell-derived hepatocyte-like cells were used to screen small molecules for hepatotoxicity using a cell viability (**A**,**C**) and a lipid accumulation (**B**,**D**) assay. Hepatocyte-like cells were differentiated in 6-well plates according to the protocol and replated onto Matrigel-coated 96-well plates at 60,000 cells per well for use in high throughput screening experiments. Hepatocyte-like cells were either left untreated (DMSO-alone) or incubated with incremental doses of the known hepatotoxic compounds: Carboplatin (Catalog # 2626), Doxorubicin (Catalog # 2252), Crizotinib (Catalog # 5119), Tacrine (Catalog # 0965), Sunitinib (Catalog # 3768), Staurosporine (Catalog # 1285), and Entacapone (Catalog # 4720). Following 48 hours in culture with compounds, cells were incubated with (**A**,**C**) Calcein AM (Catalog # 5119), a fluorescent live-cell marker, or (**B**,**D**) LipidTox™ (ThermoFisher) a fluorescent lipid stain. Cell staining was quantified using the Operetta CLS™ High Content Analysis System. Statistical significance was determined using an unpaired T-test: * = $p \leq 0.01$, ** = $p \leq 0.05$.

Conclusions

- The StemXVivo® Hepatocyte Differentiation Kit efficiently differentiates human pluripotent stem cells into hepatocyte-like cells.
- Stem cell-derived hepatocyte-like cells demonstrate liver functions, including p450 activity which is important for drug metabolism.
- Stem cell-derived hepatocyte-like cells provide an *in vitro* model to assess hepatotoxicity.
  - Hepatocyte-like cells maintain functional when replated into 96-well plates.
  - 96-well plate format enables high throughput screening of hepatotoxic drugs.
  - Known hepatotoxic compounds are dose-dependently toxic to stem cell-derived hepatocyte-like cells.
  - Fluorescent assays are available for high throughput experiments.