Modeling Hepatocyte Differentiation with Flow Cytometry Analysis

Introduction

iCell® Hepatoblasts represent a progenitor population upstream in differentiation from the iCell Hepatocytes 2.0 and iCell Hepatocytes products. This cell type reflects the point in differentiation from induced pluripotent stem (iPS) cells past endoderm but before being a fully specified hepatocyte. Thus, the hepatoblast cells retain the potential to differentiate into both hepatocytes and cholangiocytes. The protocol presented here has demonstrated utility in inducing differentiation of iCell Hepatoblasts into the hepatocyte lineage.

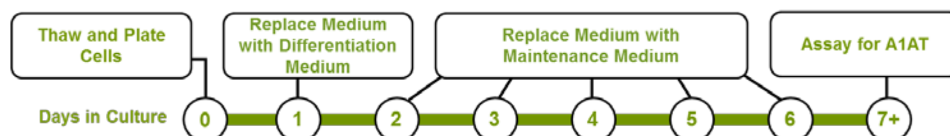
Required Equipment and Consumables

The following equipment and consumables are required in addition to the materials specified in the iCell Hepatoblasts Prototype User's Guide.

Item	Vendor	Catalog Number
Equipment		
Flow Cytometer	Multiple Vendor	
Consumables		
iCell Hepatoblasts Prototype	Cellular Dynamics International (CDI)	HBC-100-020-001-PT
Biocoat Collagen I Multiwell Plates (Cell Culture Plates)	Becton Dickinson	354408 – 24-well 354407 – 96-well
Media Components		
iCell Hepatocytes 2.0 Medium Supplement	Cellular Dynamics International (CDI)	HCS-100-021-001
B27 Supplement	Life Technologies	17504
BMP4	R&D Systems	314-BP
CHIR99021	Stemgent	04-0004-10
	Biovision	1677-25
Dexamethasone	MP Biomedicals	02190040 – white powder 02194561 – cell culture reagent, white powder
	Sigma	D8893
FGF10	R&D Systems	345-FG
FGF Basic (bFGF)	R&D Systems	233-FB
Gentamicin	Life Technologies	15750
HGF	R&D Systems	294-HGN
Oncostatin M	R&D Systems	295-OM
RPMI	Life Technologies	11875

Workflow

iCell Hepatoblasts are thawed and plated according to the iCell Hepatoblasts Prototype User's Guide. On day 1 post-plating, spent medium is replaced with Hepatocyte Differentiation Medium. On day 2 post-plating, spent medium is replaced with Hepatocyte Maintenance Medium, and spent medium is replaced daily thereafter. From day 7, differentiated hepatocytes can be labeled for alpha-1 antitrypsin (A1AT) for detection.



Methods

Thawing and Plating iCell Hepatoblasts

Thaw, plate, and maintain iCell Hepatoblasts according to the User's Guide until day 1 post-plating.

Preparing Media

Prepare and store the Hepatocyte Differentiation Medium and Hepatocyte Maintenance Medium as follows:

Hepatocyte Differentiation Medium[1,2,3]		
Component	**Amount (ml)**	**Final Concentration**
RPMI	98	98%
B27 Supplement, 50X	2	1X
BMP4, 25 µg/ml	0.2	50 ng/ml
CHIR99021, 10 mM	0.03	3 µM
FGF10, 100 µg/ml	0.06	60 ng/ml
bFGF, 100 µg/ml	0.01	10 ng/ml
Gentamicin, 50 mg/ml	0.05	25 µg/ml
HGF, 25 µg/ml	0.08	20 ng/ml

1 Prepare the Hepatocyte Differentiation Medium immediately before use.
2 Filter the medium using a 0.2 µm PES filter unit.
3 Store the Hepatocyte Differentiation Medium at 4°C for up to 1 week. Do not store at -20°C.

Hepatocyte Maintenance Medium[1,2]		
Component	**Amount (ml)**	**Final Concentration**
RPMI	96	96%
B27 Supplement, 50X	2	2%
Dexamethasone, 5 mM	0.002	0.1 µM
Gentamicin, 50 mg/ml	0.04	25 µg/ml
iCell Hepatocytes 2.0 Medium Supplement, 50X	2	1X
Oncostatin M, 10 µg/ml	0.2	20 ng/ml

1 Prepare the Hepatocyte Differentiation Medium immediately before use.
2 Filter the medium using a 0.2 µm PES filter unit.
3 Store the Hepatocyte Maintenance Medium at 4°C for up to 1 week. Do not store at -20°C.

Notes

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Differentiating iCell Hepatoblasts into Hepatocytes

The following protocol details the procedures for inducing differentiation of iCell Hepatoblasts into hepatocytes in a 96-well or 24-well cell culture plate. Scale volumes appropriately for other vessel formats.

1. Equilibrate the Hepatocyte Differentiation Medium at room temperature before use.
2. Approximately 24 hours post-plating iCell Hepatoblasts, aspirate the spent medium and replace (100%) with the appropriate volume of Hepatocyte Differentiation Medium. Recommended volumes are as follows:
   - **24-well cell culture plate:** 0.5 ml/well
   - **96-well cell culture plate:** 100 µl/well
3. Return plates to a cell culture incubator at 37°C, 5% CO_2.
4. Approximately 24 hours post-differentiation induction, aspirate the spent medium and replace (100%) with the appropriate volume of Hepatocyte Maintenance Medium.
5. Replace 100% of the spent Hepatocyte Maintenance Medium daily until day 7 post-plating.

Evaluating Differentiated Hepatocytes

From day 7 post-plating, the cell culture can be evaluated for hepatocyte purity by measuring A1AT expression by flow cytometry or imaging analysis.

- For flow cytometry analysis instructions, see the iCell Hepatocytes Application Protocol: Labeling Hepatic Markers: Alpha-1 Antitrypsin Labeling with Flow Cytometry Analysis available online at www.cellulardynamics.com/lit/.
- For imaging analysis instructions, see the iCell Hepatocytes Application Protocol: Immunofluorescent Labeling available online at www.cellulardynamics.com/lit/.

Data Analysis

See the guide for the flow cytometry system for data analysis instructions.

1. Use the isotype control sample to set the negative population gates.
2. Use the live/dead signal and A1AT signal to evaluate hepatoblast differentiation into hepatocytes (Figure 1).

Notes

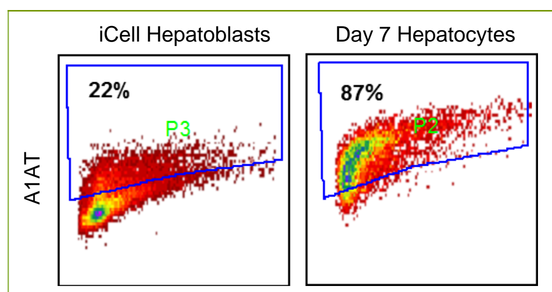

Figure 1: Flow Cytometry Analysis of A1AT Expression in Hepatocytes Differentiated from iCell Hepatoblasts
In this representative experiment, iCell Hepatoblasts are differentiated into hepatocytes as indicated by the increase in A1AT expression in the induced condition. Acquisition and analysis were performed using a BD Accuri C6 Flow Cytometer (BD Biosciences).

Summary

iCell Hepatoblasts are derived from human iPS cells and provide an in vitro cellular system for modeling hepatic development. The methods and data presented here highlight a reproducible cell culturing protocol for inducing hepatocyte differentiation monitored by the expression of A1AT by flow cytometry.

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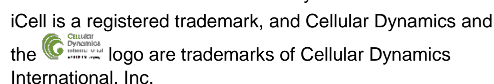
iCell is a registered trademark, and Cellular Dynamics and the  logo are trademarks of Cellular Dynamics International, Inc.
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Version: August 2016
AP-HBCHEP160805