Modeling Adipocyte Differentiation

Introduction

The ability to differentiate into multiple lineages is a fundamental characteristic of mesenchymal stem cells. iCell® Mesenchymal Stem Cells, human induced pluripotent stem cell (iPSC)-derived mesenchymal stem cells, recapitulate the physiological characteristics of native human mesenchymal stem cells. Due to their human origin, high purity, functional relevance, and ease of use, iCell Mesenchymal Stem Cells represent an optimal in vitro test system for interrogating mesenchymal stem cell multiple lineage differentiation in basic research and many areas of regenerative biology.

The Application Protocol presented here has demonstrated utility in inducing differentiation of iCell Mesenchymal Stem Cells into adipocytes as assessed by Oil Red O staining.

Required Equipment and Consumables

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

Item	Vendor	Catalog Number
Equipment		
Bright Field Microscope	Multiple Vendors	
Consumables		
iCell Mesenchymal Stem Cells Prototype	Cellular Dynamics International (CDI)	MSC 301-010-001-PT
3-isobutyl-1-methylxanthine (IBMX)	Sigma	I5879-100MG
6-well Cell Culture Plates	Multiple Vendors	
Acetone	Sigma	A949
Alpha-MEM	Gibco	11900-024
Buffered Formaldehyde	Fisher Scientific	SF93-4
Dexamethasone	Sigma	D8893
Distilled Water	Multiple Vendors	
Dulbecco's Phosphate Buffered Saline without Ca^{2+} and Mg^{2+} (D-PBS)	Life Technologies	14190
Ethanol	Fisher Scientific	BP2818500
Fetal Bovine Serum (FBS)	Hyclone	SH30071.03
Filter Paper	Fisher Scientific	09-795F
Hematoxylin*	Sigma	MHS-1
Indomethacin	Sigma	I7378-5G
Insulin, Recombinant Human	Sigma	I9278-5ML

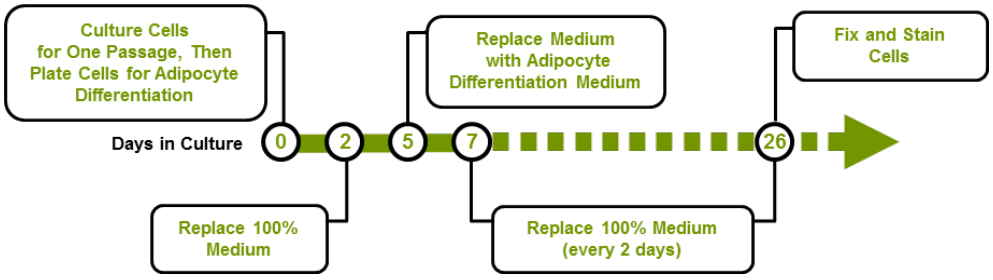
Item	Vendor	Catalog Number
Oil Red O	Sigma Aldrich	O0625
Penicillin/Streptomycin	Multiple Vendors	

* Optional. This protocol presented here provides instructions for staining with O Red O and optionally with hematoxlyn.

Notes

Workflow

iCell Mesenchymal Stem Cells are thawed and plated into a tissue culture treated plate. When iCell Mesenchymal Stem Cells reach 80% - 90% confluency (approximately day 5), cells are passaged and plated for adipocyte differentiation. When iCell Mesenchymal Stem Cells reach confluency, 100% of spent medium is replaced with Adipocyte Differentiation Medium and every 2 days thereafter. Optimal adipocyte differentiation is observed at day 21 post-adipocyte induction.



Methods

Preparing the Adipocyte Differentiation Medium

Using sterile technique, combine the following components at the final concentrations specified to prepare the Adipocyte Differentiation Medium. Scale reagent volumes as needed.

Component	Amount (ml)	Final Concentration
IBMX[1], 45 mM	10	0.45 mM
Alpha-MEM	1,000	100%
Dexamethasone[2], 5 mM	0.02	0.1 µM
FBS	100	10%
Indomethacin[3], 50 mM	2.5	0.2 mM
Insulin, Recombinant Human	0.1	1 µg/ml
Penicillin/Streptomycin	10	1%

1 Dissolve 100 mg of IBMX in 10 ml of 96% ethanol to achieve a 5 mM stock solution.

2 Dissolve 1 mg of dexamethasone in 500 µl DMSO to achieve a 5 mM stock solution.

3 Dissolve 100 mg of indomethacin in 5.6 ml of 96% ethanol to achieve a 50 mM stock solution.

Notes

Thawing iCell Mesenchymal Stem Cells

1. Thaw iCell Mesenchymal Stem Cells according to their User's Guide.
2. Remove a sample of cells to confirm the viability using a hemocytometer (using trypan blue exclusion to identify viable cells) or an automated cell counter.
3. Dilute the cell suspension in Maintenance Medium to achieve a cell density of 35,000 cells/cm^2.

Performing the Adipocyte Differentiation

1. When iCell Mesenchymal Stem Cells reach 80% - 90% confluency, passage the cells using TrypLE according to their User's Guide, quenching the enzyme with an equal volume of Plating Medium.
2. Remove a sample of cells to perform a cell count using a hemocytometer (using trypan blue exclusion to identify viable cells) or an automated cell counter.
3. Centrifuge the cell suspension at room temperature at 400 x g for 5 minutes.
4. Carefully aspirate the supernatant, taking care not to disturb the cell pellet.
5. Resuspend the cells in the appropriate volume of Maintenance Medium to plate the cells at a density of 25,000 viable cells/cm^2.
6. Culture the cells in a cell culture incubator at 37°C, 5% CO_2.
7. When the cells reach 80% - 90% confluency (approximately day 5 - 7), replace 100% of spent medium with Adipocyte Differentiation Medium.
8. Replace the Adipocyte Differentiation Medium every 2 days. At day 21 post-adipocyte induction, the cells can be labeled for adipocyte-specific markers or used for downstream applications.

Staining Differentiated Adipocytes

1. Prepare the Oil Red O staining solution:
   a. Dissolve 1 g of Oil Red O powder in a 1:1 mixture of 50 ml of acetone and 50 ml of ethanol.
   b. Filter the Oil Red O staining solution using filter paper.
2. Fix the cells with buffered formaldehyde:
   a. Aspirate the spent medium.
   b. Wash the cells once with 1 - 5 ml/well of D-PBS.
   c. Add 1 - 5 ml of buffered formaldehyde per well to cover the area of the cells.
   d. Incubate the plate at room temperature for 20 minutes.
3. Stain the cells with Oil Red O staining solution:
   a. Aspirate the buffered formaldehyde.
   b. Wash the cells 2 times with distilled water.
   c. Add 70% ethanol to the cells.
   d. Incubate the plate at room temperature for 1 minute.

e. Aspirate the ethanol.

f. Add 1 ml of Oil Red O staining solution to the plate.

g. Incubate the plate at room temperature for 10 - 15 minutes.

h. Aspirate the staining solution.

i. Rinse the cells with 70% ethanol.

j. Aspirate the ethanol.

k. Wash the plate 2 times with distilled water.

l. Aspirate the distilled water.

m. Visualize the cells using the bright field microscope.

4. (Optional) Stain the cells with hematoxylin:

   a. Add 1 ml of hematoxylin.

   b. Incubate at room temperature for 10 minutes.

   c. Aspirate the hematoxylin. Fill the well with cool tap water.

   d. Incubate the plate at room temperature for 15 minutes.

   e. Without removing the water, immediately visualize the cells using the bright field microscope.

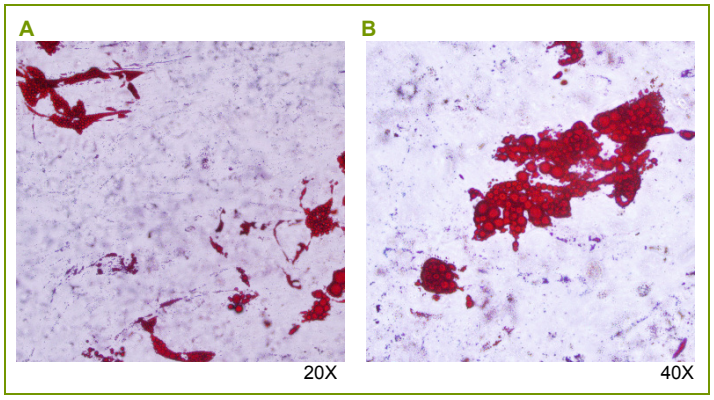


Figure 1: Differentiation into Adipocytes
In this representative experiment, iCell Mesenchymal Stem Cells differentiated into adipocytes as assessed by the presence of lipid droplets and positive Oil Red O stain.

Notes

Notes

Summary

iCell Mesenchymal Stem Cells are derived from human iPSCs and provide an in vitro cellular system for adipocyte differentiation. The methods and data presented here highlight a reproducible cell culture protocol for inducing adipocyte differentiation as assessed by Oil Red O staining.

Revision History
Version 1.0: December 2016
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