Modeling Chondrocyte 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 chondrocytes as assessed by Alcian blue staining.

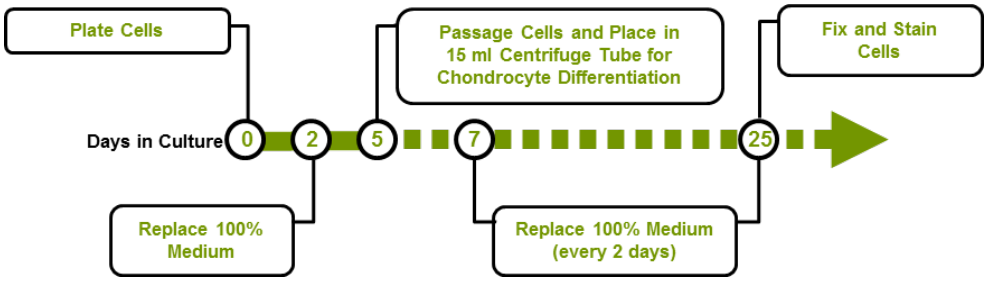
Required Consumables

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

Item	Vendor	Catalog Number
iCell Mesenchymal Stem Cells Prototype	Cellular Dynamics International (CDI)	MSC-301-010-001-PT
Alcian Blue	Millipore	SK-5300
Ascorbic Acid	Wako	013-19641
Buffered Formaldehyde	Fisher Scientific	SF93-4
Dexamethasone	Sigma	D8893
Distilled Water	Multiple Vendors	
DMEM High Glucose	Life Technologies	11960-069
Dulbecco's Phosphate Buffered Saline without Ca^{2+} and Mg^{2+} (D-PBS)	Life Technologies	14190
Glacial Acetic Acid Solution	Sigma	A0808
ITS+ Premix Tissue Culture Supplement	Corning	354352
Sodium Pyruvate	Life Technologies	11360-070
TGF-β1	R&D Systems	240-B

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 placed in a 15 ml centrifuge tube in the presence of Chondrocyte Differentiation Medium. 100% of spent Chondrocyte Differentiation Medium is replaced every 2 days. Optimal chondrocyte differentiation is observed at day 20 post-chondrocyte induction.



Methods

Preparing the Chondrocyte Differentiation Medium

Using sterile technique, combine the following components at the final concentrations specified to make the Chondrocyte Differentiation Medium. Scale the reagents as needed.

Component	Amount (ml)	Final Concentration
Ascorbic Acid, 20 mg/ml	0.014	1 µM
Dexamethasone, 5 mM	0.02	0.1 µM
DMEM High Glucose	1,000	100%
ITS+ Premix Tissue Culture Supplement	100	10%
Sodium Pyruvate	10	1%
TGF-β1, 2 µg/ml	5	10 ng/ml

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 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 Chondrocyte 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 confirm viability 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.

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4. Carefully aspirate the supernatant, taking care not to disturb the cell pellet.
5. Resuspend the cells in 15 ml centrifuge tubes at 500,000 viable cells/tube in 500 µl Chondrocyte Differentiation Medium.
6. Culture the cells in a cell culture incubator at 37°C, 5% CO_2.

   Note: *Caps on the tubes should be loose to allow for gas exchange while in the incubator.*
7. 2 days post-differentiation induction, add 500 µl of Chondrocyte Differentiation Medium per tube.
8. Replace the medium every 2 days:
   a. Centrifuge the cells at 400 x g for 5 minutes.
   b. Remove the spent medium.
   c. Resuspend the cells in 500 µl of Chondrocyte Differentiation Medium per tube.

   At day 20 post-chondrocyte induction, the cells can be labeled for chondrocyte-specific markers or used for downstream applications.

Staining Differentiated Chondrocytes

1. Prepare a 3% glacial acetic acid solution.
2. Remove the tubes from the cell culture incubator.
3. Centrifuge the chondrocyte spheroids at room temperature at 400 x g for 5 minutes to sediment at the bottom of the tube.
4. Carefully aspirate the spent medium.

   Note: *Do not let the chondrocyte spheroids to dry for longer than 30 seconds throughout the entire staining procedure.*
3. Carefully wash the chondrocyte spheroids twice with 1 - 2 ml of D-PBS, taking care not to aspirate the spheroids during the washes.
4. Fix the chondrocyte spheroids with buffered formaldehyde:
   a. Carefully aspirate the D-PBS.
   b. Add a volume of buffered formaldehyde to cover the chondrocyte spheroids.
   c. Incubate at room temperature for 30 minutes.
5. Stain the chondrocyte spheroids with Alcian blue:
   a. Add 15 ml/tube of D-PBS to wash the chondrocyte spheroids.
   b. Centrifuge the chondrocyte spheroids at 400 x g for 5 minutes.
   c. Carefully aspirate the D-PBS.
   d. Wash the chondrocyte spheroids twice with distilled water.
   e. Rinse the chondrocyte spheroids with 3% glacial acetic acid solution.
   f. Centrifuge at 400 x g for 5 minutes.
   g. Aspirate the 3% glacial acetic acid solution.

h. Add a volume of Alcian blue to cover the chondrocyte spheroids.

i. Incubate at room temperature for 5 - 10 minutes.

   Note: *For large chondrocyte spheroids, increase the incubation time an additional 5 minutes.*

j. Centrifuge the chondrocyte spheroids at 400 x g for 5 minutes.

k. Carefully aspirate the Alcian blue.

l. Quickly wash the chondrocyte spheroids with 1 ml of 3% glacial acetic acid solution.

m. Centrifuge the chondrocyte spheroids at 400 x g for 5 minutes.

n. Carefully aspirate the 3% glacial acetic acid solution.

o. Wash the chondrocyte spheroids twice with 5 ml of D-PBS.

p. Centrifuge the chondrocyte spheroids at 400 x g for 5 minutes.

Alcian blue stains the entire chondrocyte spheroids. To visualize the architecture of the developing chondrocytes, it is recommended to fix and embed the chondrocyte spheroids in specimen processing gel for routine tissue processing, paraffin embedding, microtomy, deparafinazation, and staining the cross sections of chondrocytes with Alcian blue.

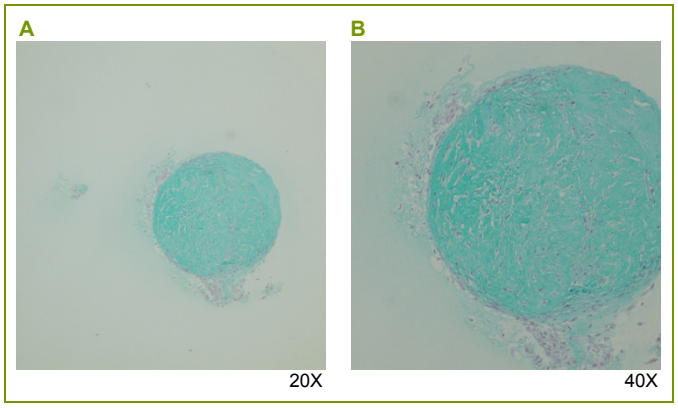

Figure 1: Differentiation into Chondrocytes
In this representative experiment, iCell Mesenchymal Stem Cells differentiated into chondrocytes. Cross sections of chondrocytes stained positive by Alcian blue stain.

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Summary

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

Revision History
Version 1.0: December 2016
AP-MSCCHO161201