Stem Cell Culture & Adipogenic Differentiation Protocol

Description

This protocol describes methods to culture human stem cells in collagen hydrogels and induce the cells into mature adipocytes. Cell differentiation is characterized and detected by Oil Red O staining on hydrogel cultures and cells cultured in 2D.

Materials Needed

- PureCol® (Cat #5005)
- Stem cells
- Cell culture media with serum and antibiotics
- Cell culture flasks
- Trypsin-EDTA (Fisher Scientific, Cat# 25-200-114)
- Dexamethasone (DEX) (Sigma -Aldrich, Cat# D2915-100MG,)
- 3-Isobutyl-1-methylxanthine (IBMX) (Sigma -Aldrich, Cat# I5879-5G)
- Indomethacin (IM) (Sigma -Aldrich, Cat# I7378-25G)
- 4% paraformaldehyde (ThermoFisher, Cat # 047392-9M)
- Oil Red O (Sigma-Aldrich, 198196)
- Isopropanol (Sigma-Aldrich, 109827)
- Syringes
- 0.22 µm filters
- DI water
- Hemocytometer
- Cell culture plates
- Serological pipettes
- Eppendorf tubes

Procedure

Preparation of Adipogenic Supplements (A.S.) Stock Solutions

- **1 mM DEX stock:** Dissolve 0.0196 g Dexamethasone in 50 mL of DI Water.
- **30 mM IBMX stock:** Dissolve 0.334 g 3-Isobutyl-1-methylxanthine in 50 mL of DI Water.
- **5 mM IM:** Dissolve 0.09 g Indomethacin in 50 mL of DI Water.

1. Vortex to mix the powder in water.
2. Filter the stock solution through 0.22 µm filters.
3. Aliquot the stock solution into 1.5 mL Eppendorf tubes.
4. Store the aliquoted stockat -20°C for long-term storage.

Cell culture and differentiation

1. Culture cells in PureCol® hydrogel following the Hydrogel Cell Culture Protocol. Culture cells in well-plates as 2D controls.
2. Replace the cell culture medium periodically and closely monitor cell confluency.
3. Start differentiation when the cells reach around 70% confluency. Replace the cell culture medium with the conditioned medium to induce cell differentiation.

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Table 1. Conditioned medium compositions for different volumes

Medium (mL)	100	200	300	400	500
DEX (µL)	50	100	150	200	250
IM (µL)	1	2	3	4	5
IBMX (µL)	167	334	501	668	835

*Directly use DEX and IM stock and add to the medium.
**Dilute IBMX stock in PBS (60 µL IBMX in 940 µL PBS) before adding it to the medium.

4. Culture the cells with the conditioned medium for up to 28 days.

5. Fix the samples with 4% paraformaldehyde for staining and imaging.

Staining solution preparation

1. Add 0.25 g Oil Red O powder into 50 mL isopropanol and dissolve the powder completely to make the 0.5% Oil-Red-O stock solution. Store the stock solution in a tightly capped bottle/tube at room temperature.

2. Mix 3 parts 0.5% Oil-Red-O Stock and 2 parts of PBS to prepare the fresh working solution. Mix the solutions well and incubate for 10 minutes.

3. Filter the working solution through a sterile filter unit. The working solution is ready to use.

Staining for hydrogels

1. Rinse the fixed hydrogel with PBS twice.

2. Gently wash the hydrogel with 60% isopropanol once.

3. Add excess Oil Red O working solution to the wells to cover the hydrogel completely.

4. Incubate the samples at room temperature for 1 hour, then discarding the staining solution.

5. Wash the hydrogel once with 60% isopropanol.

6. Wash the hydrogel with PBS thrice to remove excessive staining solution. Perform each wash for 5 to 8 min. Aspirate the PBS after each wash and replace with fresh PBS. If necessary, continue washing until the background is clear.

7. Add fresh PBS to the hydrogel and proceed to imaging. Image the sample under an inverted microscope with a colored bright field filter.

Staining for 2D cell controls

1. Aspirate the cell culture media and rinse each well with 2 mL PBS (cells cultured in 6 well-plate).

2. Fix the cells with 4% paraformaldehyde at room temperature for 1 hr.

3. Remove the fixative and rinse with PBS three times.

4. Add 1 mL of Oil Red O working solution to each well.

5. Incubate for 20 mins at room temperature and aspirate.

6. Rinse the wells stained with Oil Red O with 1 mL of PBS and aspirate.

7. Repeat Step 7 two times or until the background/well is clear.

8. Add a final 1 ml of PBS to each Oil Red O stained well and proceed to image.

   NOTE: 2D cultures can be kept in this condition for extended periods to allow for microscopic analysis. Add more PBS to limit evaporation.

Notes:

1. The Oil Red O working solution needs to be discarded after each experiment and cannot be reused for staining. Prepare fresh working solution for each new experiment.

