BME 174 Week 5: Verification of Adipogenicity & Myogenicity

2.15.2024

General Notes

* Typically, immunostaining is performed to verify myosin-heavy-chain (MHC) in muscle cell cultures. Because this is a two-day process and requires a fluorescent microscope, we are using alternative methods to view myotubes
* Because the cells have been fixed, we are no longer worried about contamination and can do everything outside of the biosafety cabinet
* LADD stain is a blue/purple stain we can use to visualize cells/tissues. Proliferating cells should have a light cells and dark nuclei, while differentiated myotubes should have dark staining in the tubes and light staining in the nuclei
* Oil Red O is a red stain for neutral lipids that can be both visualized with light microscopy or quantified in a plate reader by eluting the dye with isopropanol

Materials

* 70% ethanol (*used by instructors to fix cells*)
* 4% Paraformaldehyde (*used by instructors to fix cells*)
* PBS
* LADD stain
* Oil Red O
* Propylene Glycol
* Hematoxylin
* Distilled water
* 96-well plate
* Waste beaker
* Heat block/water bath set to 60C
* Plate reader
* Phase-contrast light microscope

Method

LADD Stain (for **muscle cells**)

*Done previously by instructors:*

1. Carefully aspirate media from each well of your muscle cells (4x wells in a 12-well plate)
2. Add 1 mL of PBS to each well (avoid directly pipetting liquid onto the culture, instead try to add it to the side of the well to avoid disrupting your cultures)
3. Remove the PBS
4. Add 500 uL 70% ethanol to each well for 10 minutes to fix cells
5. Aspirate 70% ethanol, replace with PBS and store in the fridge

*To do in class:*

1. Carefully remove the PBS by pipetting it into the waste beaker
2. Add 500 uL LADD stain for 60 seconds – check that this covers the bottom of the well. If not, add more.
	1. *LADD will stain the benchtop/anything it comes into contact with; please be careful to not drip/spill!*
3. Remove LADD, wash with 1 mL distilled water (i.e., pipette water into the well and then out and into the waste beaker) until the water no longer looks purple
4. Add 500 uL PBS to each well
5. View cells under the light microscope, take an image with your cell phone to use for your lab report and note any observations

Oil Red O (for **fat cells**)

*Done previously by instructors:*

1. Fix cells in 4% paraformaldehyde for 30 minutes (in fume hood)
	1. Aspirate media and rinse twice with prewarmed 1x PBS
	2. Pipette onto side of wells to not disrupt adherent cells with direct contact
2. Rinse 3x with warm 1x PBS to remove any remaining paraformaldehyde, store in fridge

*To do in class:*

1. Heat Oil Red O solution in preheated 60C water bath/heat block
2. Pipette out PBS and add 1 mL propylene glycol for 5 minutes
3. Remove propylene glycol and add 1 mL of heated (60C) Oil Red O Solution for 7 minutes
4. Prepare a solution of 85% propylene glycol in distilled water
5. Remove Oil Red O and add 1 mL 85% propylene glycol for 1 minute
6. Rinse each well twice with distilled water (carefully pipette to remove)
7. View cells under the light microscope, take an image with your cell phone to use for your lab report and note any observations

*Quantitative assessment of the degree of staining*

1. After imaging, take your plate to the fume hood (ask an instructor to help)
2. Add 500 uL isopropanol to each well to elute the Oil Red O
3. Immediately collect the isopropanol and transfer to a 96-well plate
	1. The entire class will share one 96-well plate, so note where your group loaded your samples on the plate
	2. Load some wells with isopropanol as a blank reading
4. Read optical density at 540 nm on a plate reader (*Instructors will do this once everyone has loaded their samples*)
5. Take picture of plate results

References

 McColl, R., Nkosi, M., Snyman, C., & Niesler, C. (2016). Analysis and quantification of in vitro myoblast fusion using the LADD Multiple Stain. BioTechniques, 61(6), 323-326.