BME 174 Week 8: Analysis of DOE conditions with Oil Red O/Prep 3D constructs

February 14, 2024

General Notes

* 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
* Two weeks ago, you prepared T-flasks with fat cells and allowed them to differentiate. Last week, you identified a material that you’re interested in testing the mechanical properties of. Today, you will set up an experiment to test the hypothesis that adding fat cells to your material will change the mechanical properties.
* Two weeks ago, you also set up a 48-well plate and have been feeding with various media formulations for the DOE. Today, you will use ORO to determine (1) what media formulation is optimal and (2) if there are any interactions between the 4 different media additives
* For both parts of class, you can work outside the biosafety cabinet (on the bench) because we do not plan to further culture cells and thus do not need to work in a sterile environment

Materials

Oil Red O

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

Hydrogel Experiments

* 2% (w/v) Alginate Solution
* 5% (w/v) CaCl2 Solution (concentrated stock)
* PBS
* Distilled Water
* Parafilm

Method

Oil Red O

*Done on previously by instructors:*

1. Fix cells in 4% paraformaldehyde for 30 minutes
   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 150 uL propylene glycol to each well for 5 minutes
   1. Make sure to be VERY careful when pipetting liquid into/out of the wells as to not detach the fat cells!
3. Remove propylene glycol and add 150 uL of heated (60C) Oil Red O Solution per well for 7 minutes
4. Remove Oil Red O and add 150 uL 85% propylene glycol per well for 1 minute
5. Rinse twice with distilled water
6. 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 110 uL isopropanol to each well to elute the Oil Red O
3. Collect 100 uL isopropanol and transfer to a 96-well plate (we added more than we remove to account for liquid lost during pipetting)
   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

Setup of Mechanical Testing Experiment:

1. Collect/prepare the materials for protocol determined in week 7 (CaCl2 and alginate)
   1. Make enough for 6 samples: 3 samples containing cells and 3 samples without cells as a control
2. Collect cells from T-flask
   1. Gently aspirate media from T-flask and place into communal media waste bottle
   2. Using a cell scraper, remove fat cells from flask.
      1. Start with the cell scraper at the back of the flask, and gently pull toward you. You should see the fat cells detach from the bottom of the flask
3. Mix fat with alginate in a 1:1 (volume:volume) ratio
   1. Do your best to approximate this. Feel free to ask for help!
4. Split alginate/fat mixture into 3 even samples (in separate wells of a well plate)
   1. Crosslink hydrogel as defined last class
5. Prepare 3 “control” hydrogels that contain no fat (in separate wells of the same plate)
   1. Aim to make these similar in dimension to the fat-containing hydrogels
6. Store hydrogels in liquid chosen in last class
7. Wrap well plate in parafilm, label, and store in fridge.

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.