Week 7: Material Testing and Fat Growth

March 7th, 2024

General Notes

* Today is broken into two parts: Continue feeding fat cell flasks and plates (variable media for DOE). Testing different protocols to produce hydrogels for 3D fat constructs.

Materials

*All parts*

* Misc. Consumables (pipettes, falcon tubes, flasks, etc.)
* Growth Medium (prepared previously)

*Feeding*

* DM2-Induction Medium (prepared previously)
* DM2-Accumulation Media (prepared previously)
	+ Large volume for flask
	+ 7 small volumes for plate

*Hydrogel Experiments*

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

Methods

Feeding Fat

1. Feed fat construct (T175) with 25mL of appropriate medium
	1. Growth Medium if still <90% confluence
	2. DM2-IM for first differentiation feeding
	3. DM2-AM for subsequent differentiation feedings
2. Feed 48-well plate with 250uL of appropriate media
3. Continue to feed flask and plate on the Sun,Tues,Thurs cycle

Material Testing

1. Alginate hydrogels can be formed by combining the alginate and CaCl2 solutions.
2. The CaCl2 solution may be diluted before use. Look in literature for reference values.
3. Determine metrics for desirability.
4. Choose at least 3 variables to test, and set up separate experiments for deciding hydrogel protocol.
	1. Initial test may provide insight for subsequent experiments. Record full process.
	2. Instructors may provide ideas for variables, but feel free to come up with your own!
5. For any long-term experiments (>3h), be sure to store hydrogels in liquid medium in well plate and seal with parafilm (to prevent dehydration).
6. Have fun investigating!

References

[1] L. Q. Wan, J. Jiang, D. E. Arnold, X. E. Guo, H. H. Lu, and V. C. Mow, “Calcium Concentration Effects on the Mechanical and Biochemical Properties of Chondrocyte-Alginate Constructs,” *Cell Mol Bioeng*, vol. 1, no. 1, pp. 93–102, Mar. 2008, doi: [10.1007/s12195-008-0014-x](https://doi.org/10.1007/s12195-008-0014-x).

[2] J. S. Yuen *et al.*, “Macroscale Adipose Tissue from Cellular Aggregates: A Simplified Method of Mass Producing Cell-Cultured Fat for Food Applications.” bioRxiv, p. 2022.06.08.495192, Jun. 18, 2022. doi: [10.1101/2022.06.08.495192](https://doi.org/10.1101/2022.06.08.495192).