

# BME 174 – Week 8

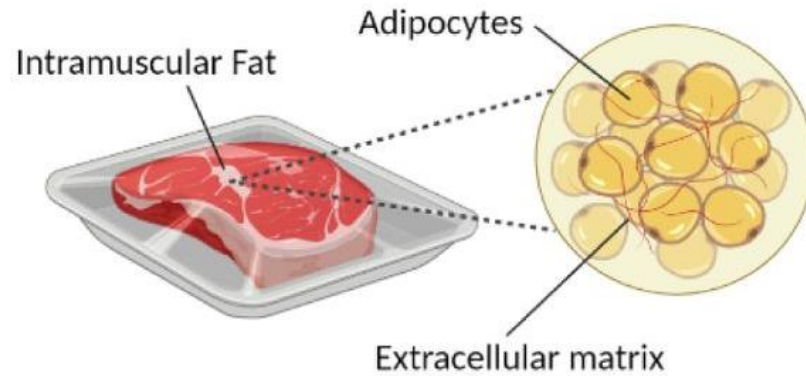
## Spring, 2023



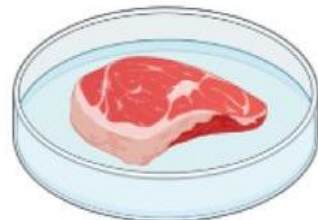
# Overview of Module 2

- Two objectives
  - To optimize the lipid accumulation media with design of experiments
  - To grow a larger amount of pig adipocytes then aggregate them into tangibly large cultured fat tissues/constructs
- Ideally, we'd first optimize media then use that for the fat tissue construct
- But because of time constraints, these two objectives will be performed separately

Yuen et al. 2023



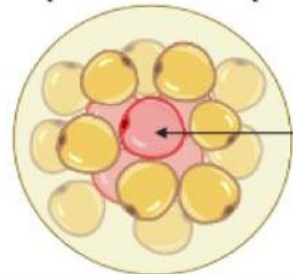
- Meat with  $\uparrow$  intramuscular fat scores higher during sensory evaluations
- Intramuscular fat is responsible for species-specific flavors



Thus, including adipose in cultivated meat is important for achieving optimal flavor

$\geq 0.5$  mm

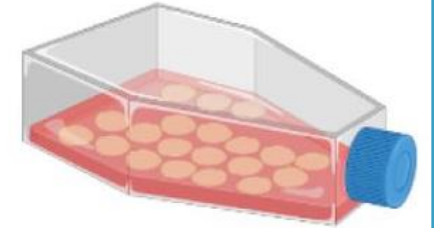
### Obstacle



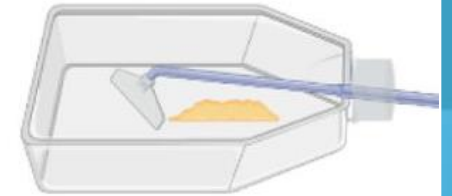
Necrotic interior during macro-scale 3D cell culture that is more than a few hundred microns thick

**Concept:** Grow adipocytes separately and aggregate them together after cell culture and lipid accumulation. This circumvents mass transport/diffusion limitations, allowing for the production of macroscale (large) cultured fat.

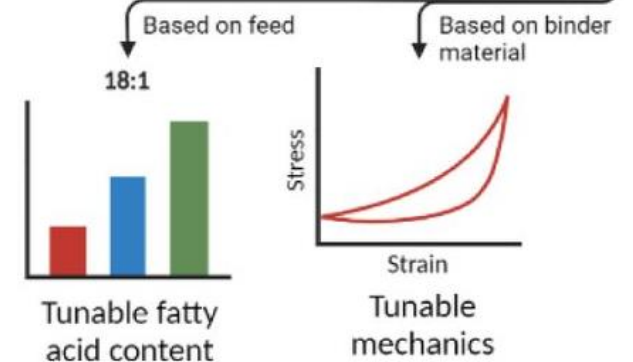
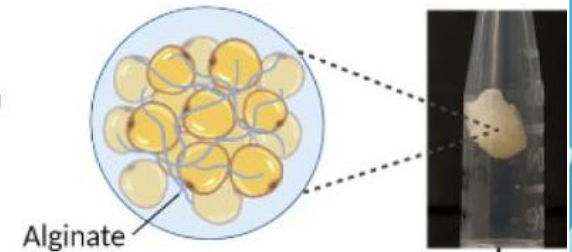
1) Obtain individual adipocytes for aggregation (e.g., 2D culture - easy cell access to nutrition)



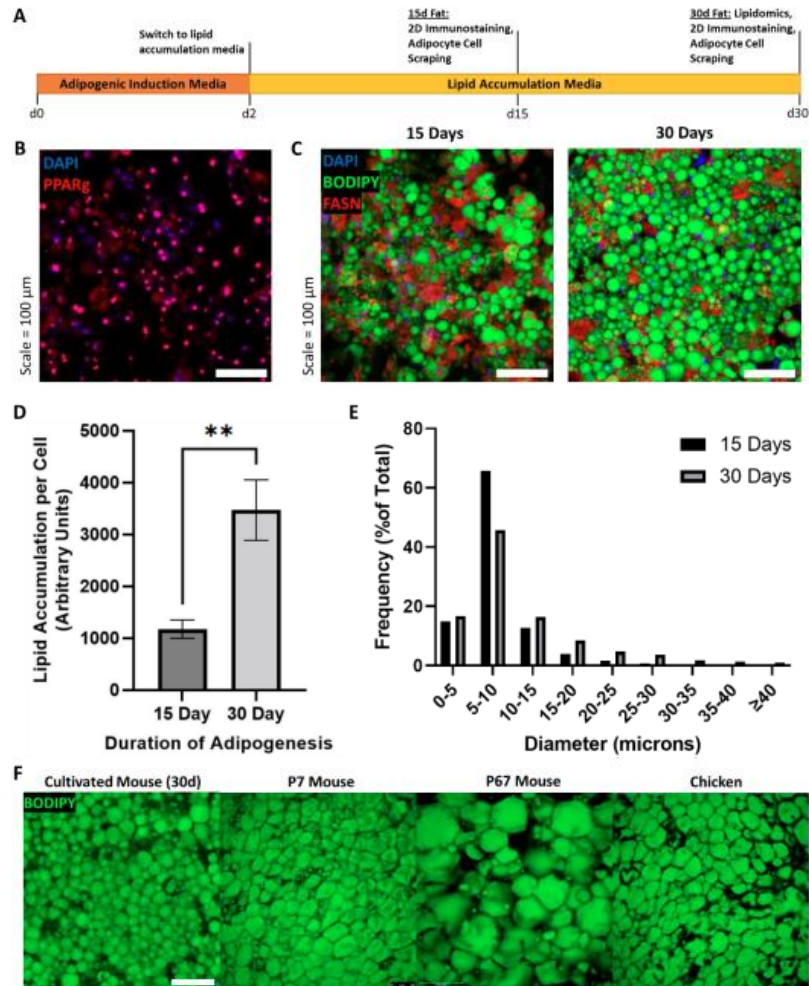
2) Harvest adipocytes and mechanically aggregate into macroscale fat



3) Maintain aggregation with a food grade binder (e.g., alginate, transglutaminase)



# Yuen et. al 2023



PPAR-gamma is activated

Cells accumulate lipids over 30 days

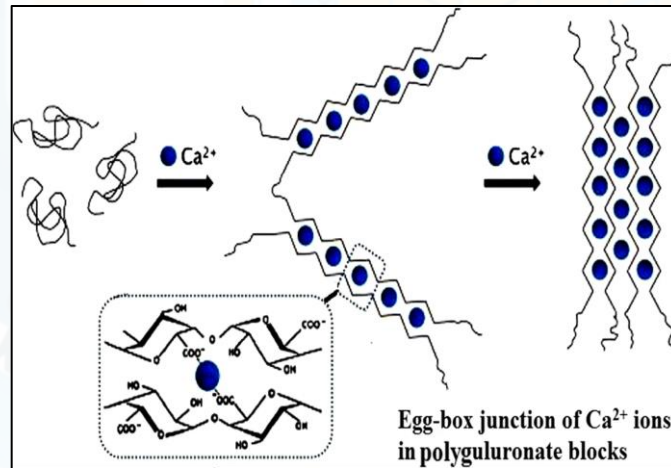
Lipid accumulation per cell increases over time

Lipid size increases over time

Lipid morphology is similar to young mouse fat

# Results from last week

- What did each group decide to use for their hydrogel?
- What are methods you used to assess which was best?
- If you saw any differences between treatments, why do you think they occurred?



A. Merakchi, S. Bettayeb, N. Drouiche, L. Adour, and H. Lounici, "Cross-linking and modification of sodium alginate biopolymer for dye removal in aqueous solution," *Polym. Bull.*, vol. 76, no. 7, pp. 3535–3554, Jul. 2019, doi: [10.1007/s00289-018-2557-x](https://doi.org/10.1007/s00289-018-2557-x).

# Experiments today...



+



NOT CROSSLINKED  
YET!

PDFAT cells seeded



Induction media



Accumulation media



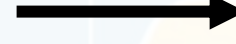
Collect lipid-filled cells  
by cell scraping

Alginate

# Experiments today...



Add  
 $\text{CaCl}_2$  to  
crosslink!

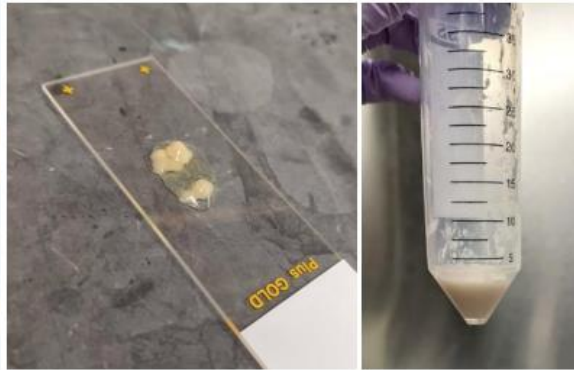
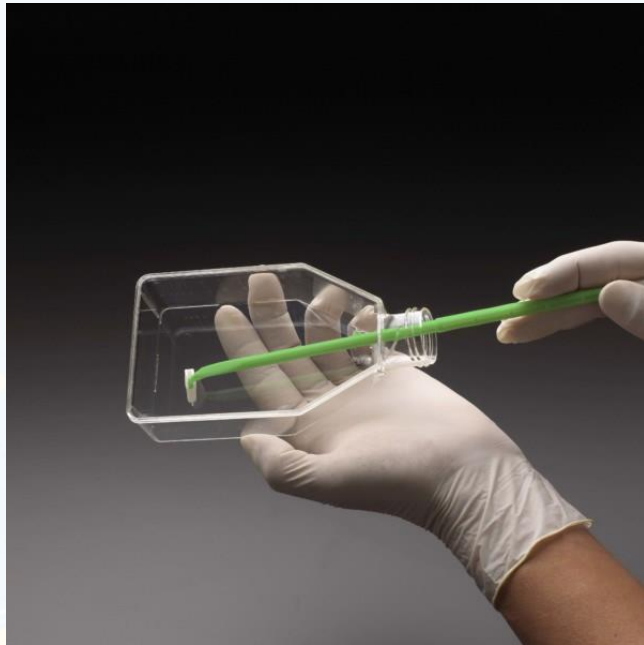


Store @ 4C



Next class: DMA!

# Key skill! Cell scraping



## Important considerations:

- If you tilt the flask, you can see what's been detached
- Be sure to get all the corners
- It's best to start at the back and carefully pull the scraper toward you



# Refresh: DOE experiment!

	1	2	3	4	5	6	7	8
A								
B		M1	M1	M1	M5	M5	M5	
C		M2	M2	M2	M6	M6	M6	
D		M3	M3	M3	M25	M25	M25	
E		M4	M4	M4				
F								

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Different groups will be assigned different media

- Group 1: #1-6, 25
- Group 2: #7-12, 26
- Group 3: #13-18, 27
- Group 4: #19-24, 28

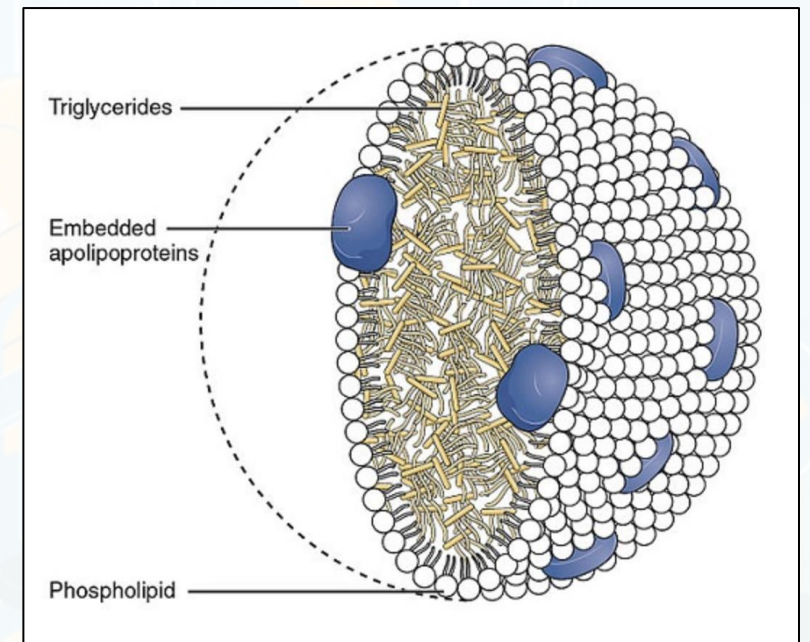
*How will we test which media leads to best lipid accumulation?*

# Oil Red O

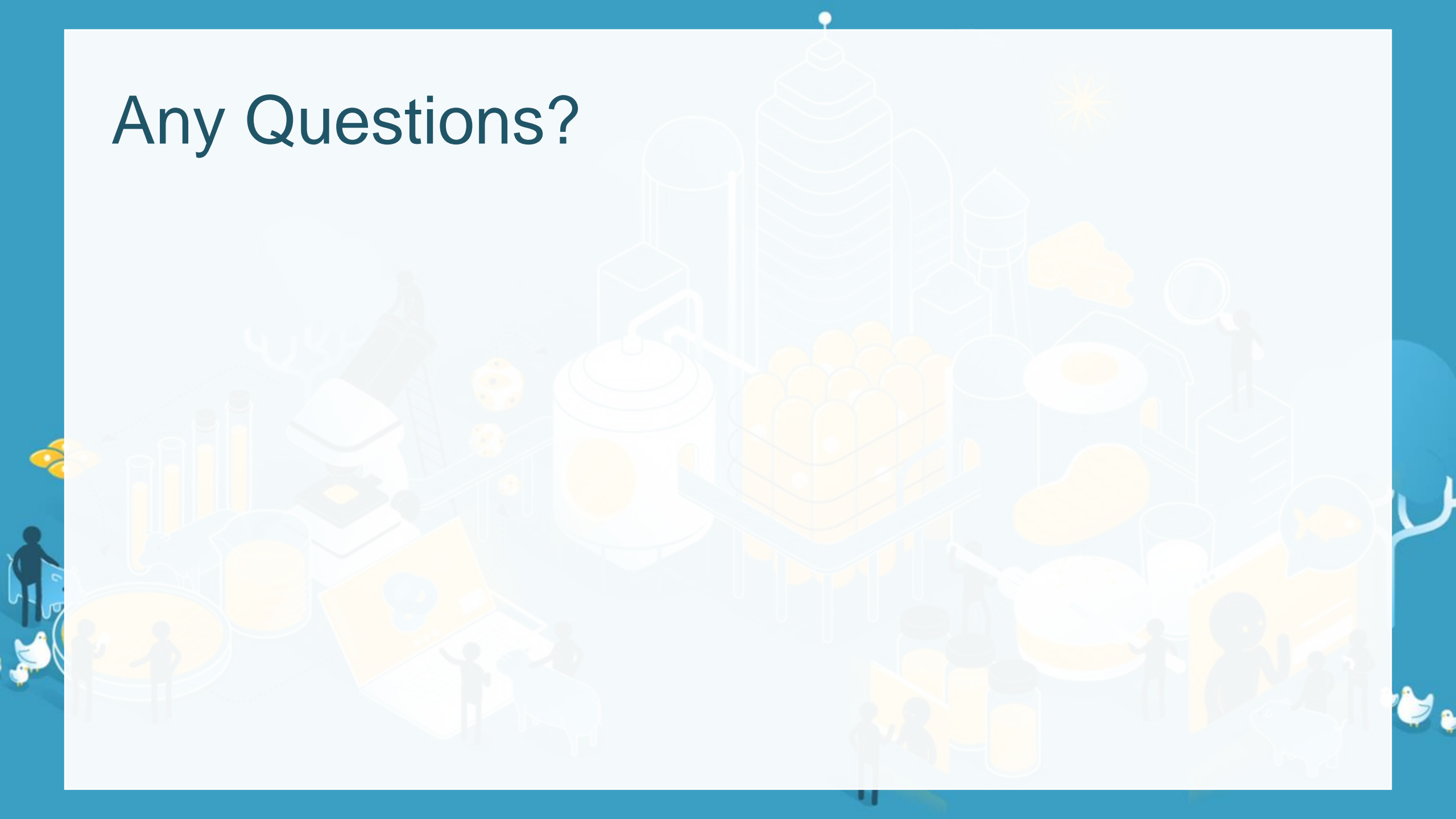
Stains **neutral lipids** via hydrophobic dye

Based on principle that ORO solubility in solvent < ORO solubility in lipids

*We will take the class' ORO data and use JMP software to determine an optimal lipid accumulation media*



# Any Questions?



# Next Week

- DMA to test mechanical properties of hydrogel + fat construct
- **Meet at 4 Colby Street Room 135**
  - Short lecture, then groups will take turns using the DMA

