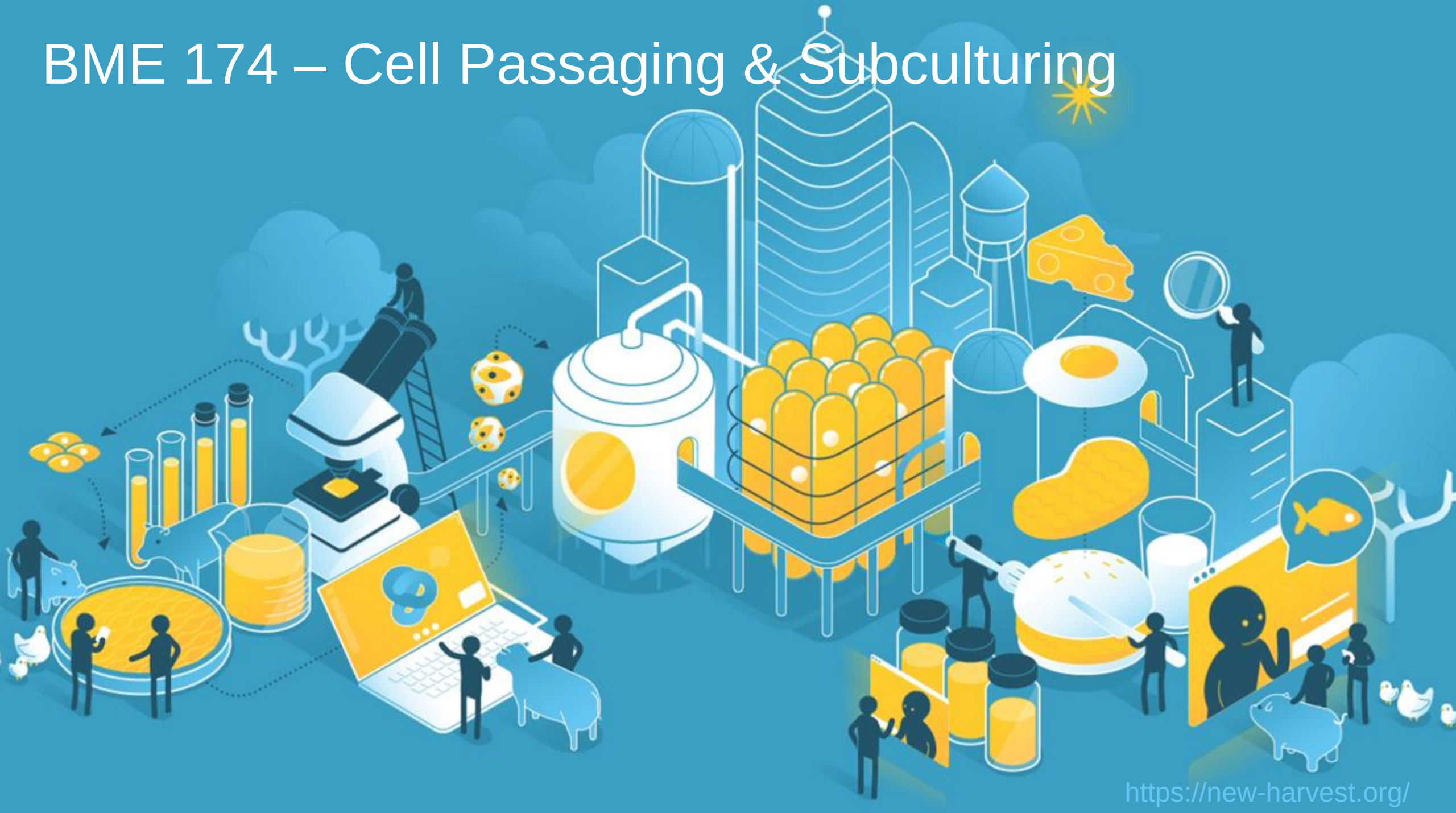
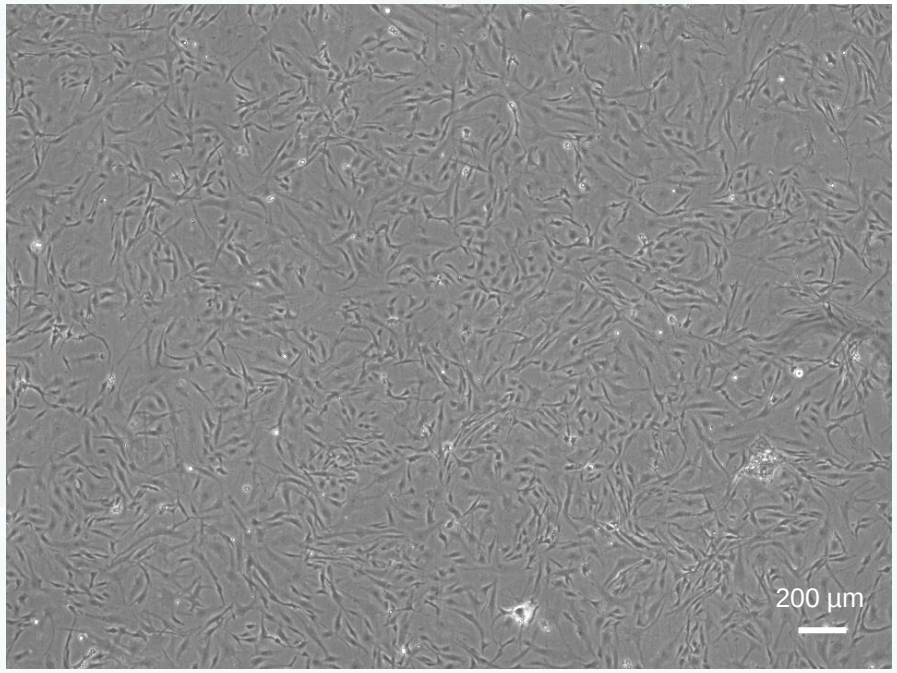
BME 174 – Cell Passaging & Subculturing

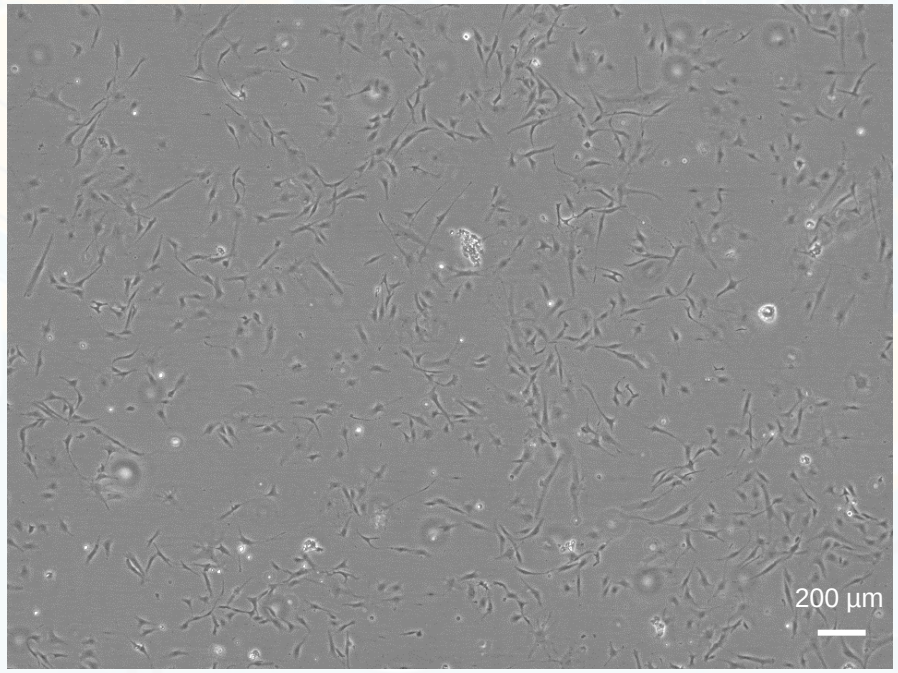


https://new-harvest.org/

Cell Passaging & Subculturing

- Cell passaging (also known as subculturing or splitting) refers to the removal of the medium and transfer of the cells from a previous culture into a new culture in fresh growth medium.



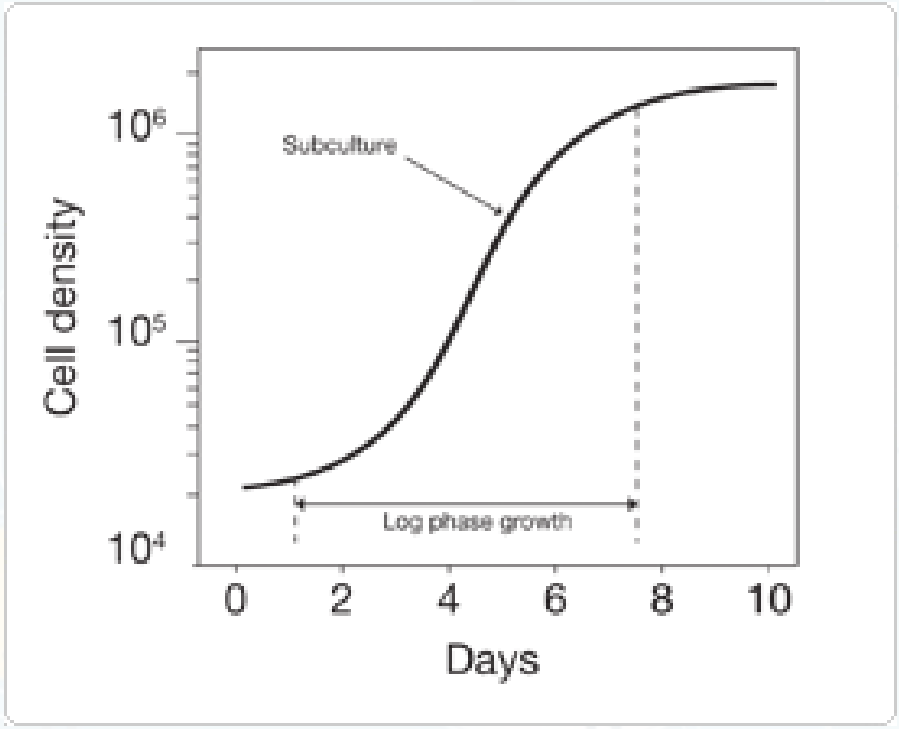


Why do we passage?

- 2 main reasons

Why do we passage?

- Maintaining log phase / avoiding growth cessation



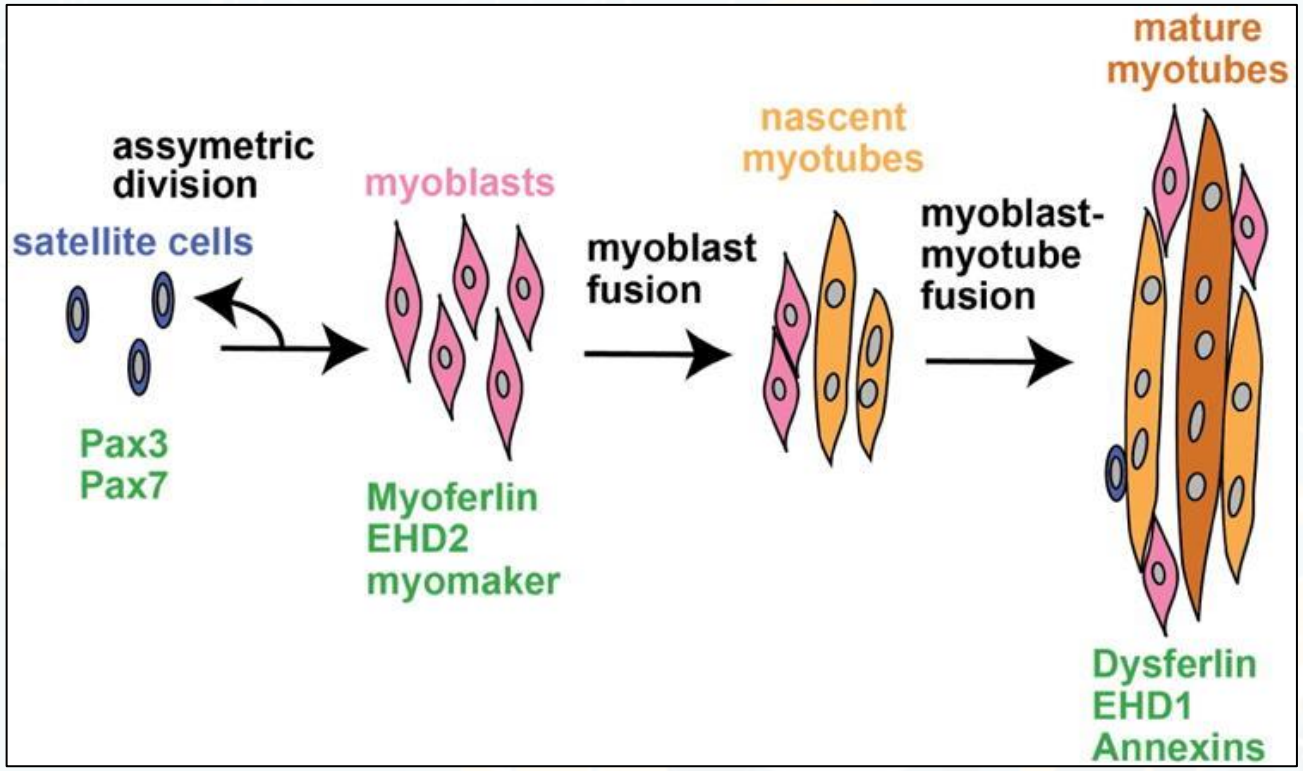
Phases of cell growth
- Lag Phase
- **Log/growth phase** ←
- Stationary phase
- (Death phase)

Aim to passage towards the end of this phase (~80% confluence)

https://www.thermofisher.com/us/en/home/references/gibco-cell-culture-basics/cell-culture-protocols/maintaining-cultured-cells.html

Why do we passage?

- Avoid differentiation
- At high cell densities, inter-cellular communication can lead to muscle differentiation → myoblast fusion



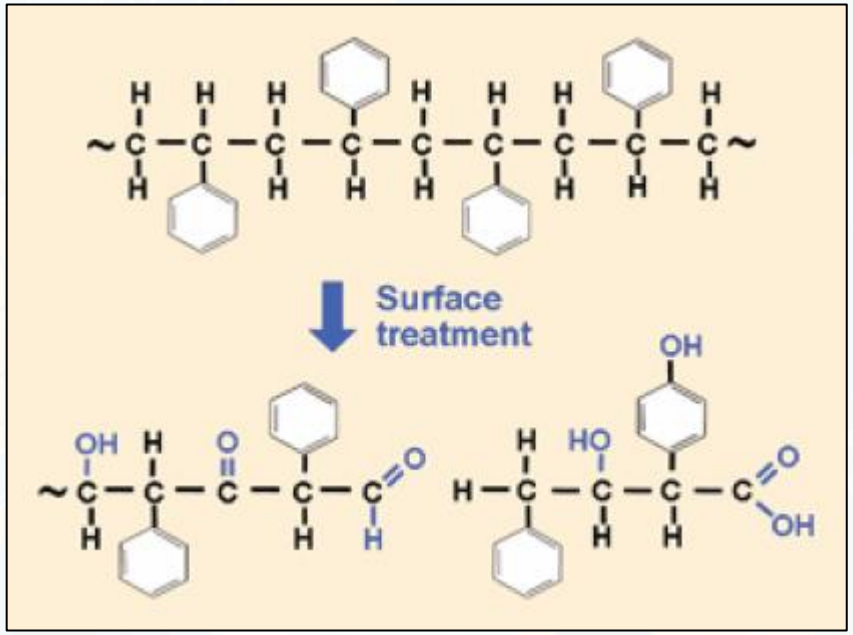
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5641474/

How do we passage?

- How are cells adherent?
- What is tissue-culture treated plastic?

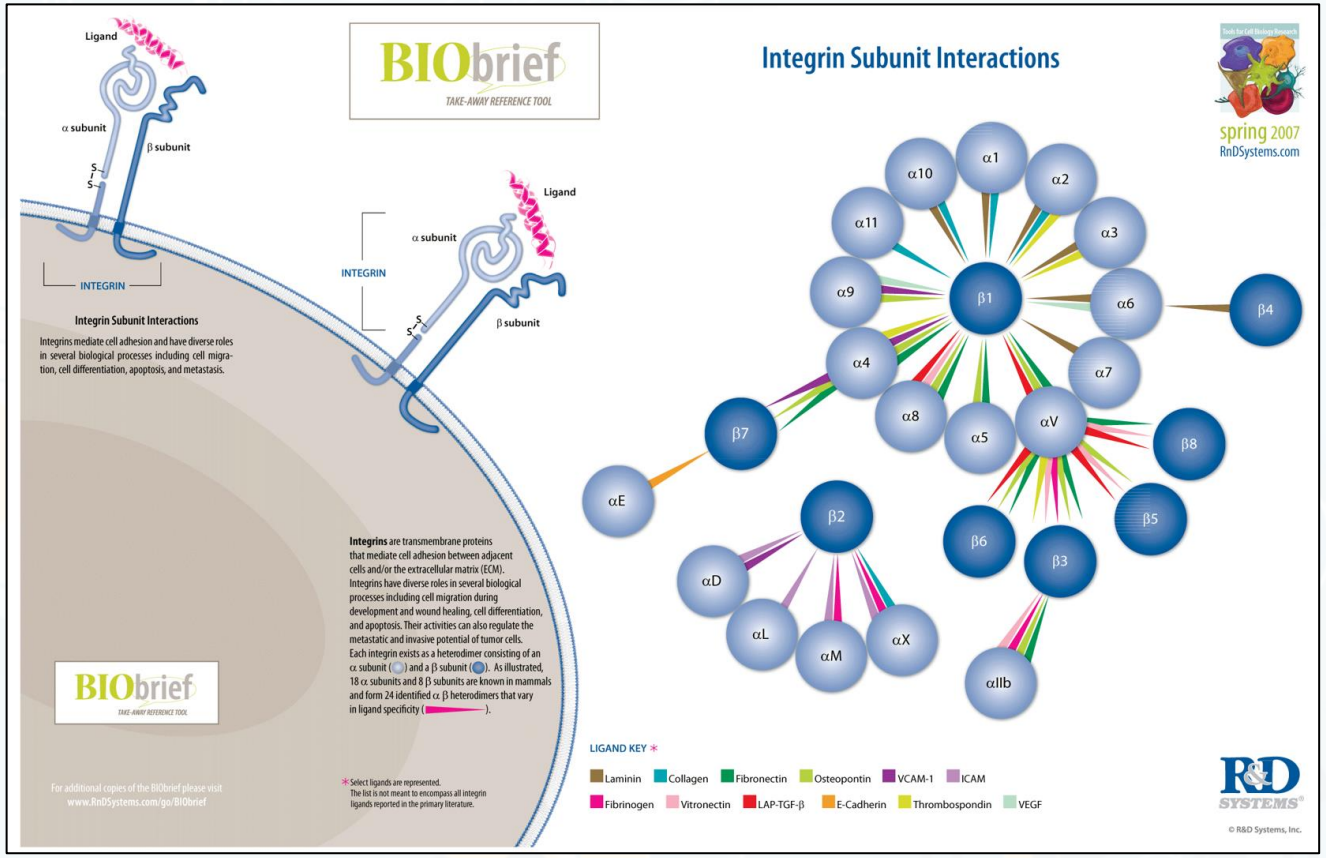
What is tissue culture treated plastic?

- Sigma's "Evolution of Cell Culture Surfaces" is a nice primer
- Tissue culture treated plastic – plasma treated to incorporate more oxygen onto the polystyrene surface
  - Increases hydrophilicity and stability
  - Promotes adsorption of extracellular matrix proteins (where do the ECM proteins come from?)



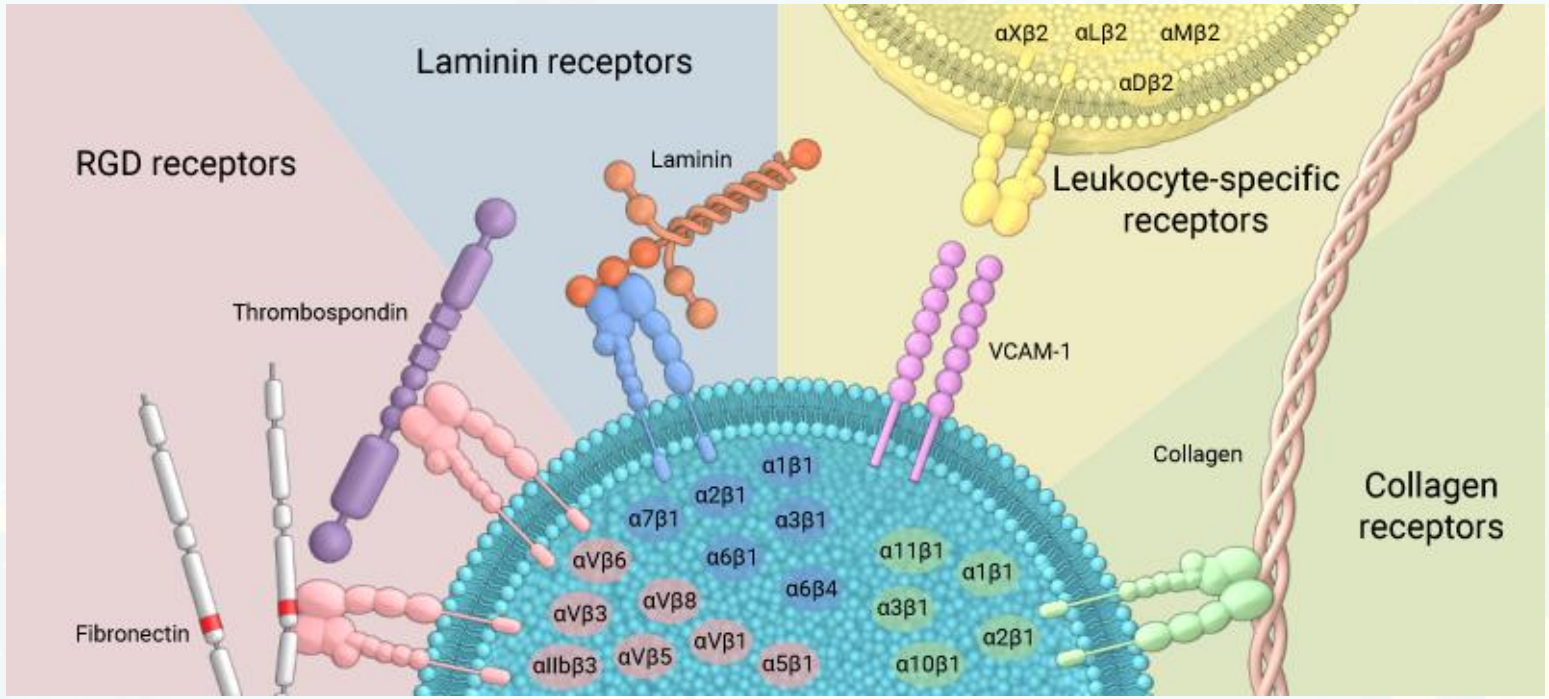
How do cells adhere?

- Integrins: transmembrane protein receptors (homodimer) that promote adhesion between the ECM and actin cytoskeleton
- Different cells/species have different integrin structures (mammals have 18 α and 8 β subunits)

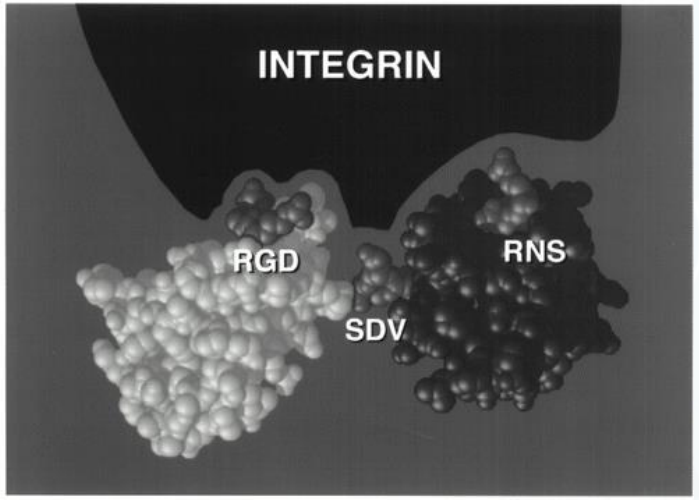


Why do integrins bind ECM?

- Through amino acid features such as “RGD” sequences



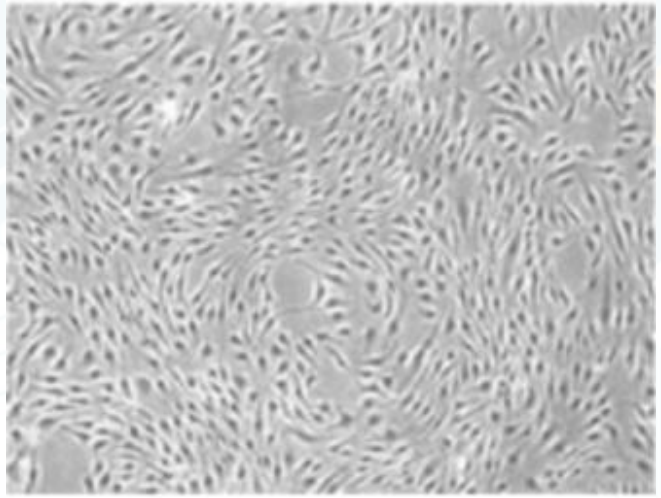
https://www.mechanobio.info/what-is-mechanosignaling/what-is-the-extracellular-matrix-and-the-basal-lamina/what-is-integrin/what-ligands-bind-to-integrin/

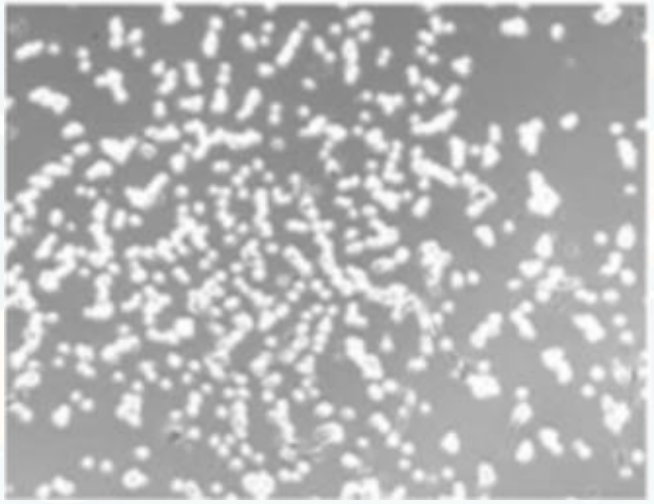


https://www.annualreviews.org/doi/10.1146/annurev.cellbio.12.1.697?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed

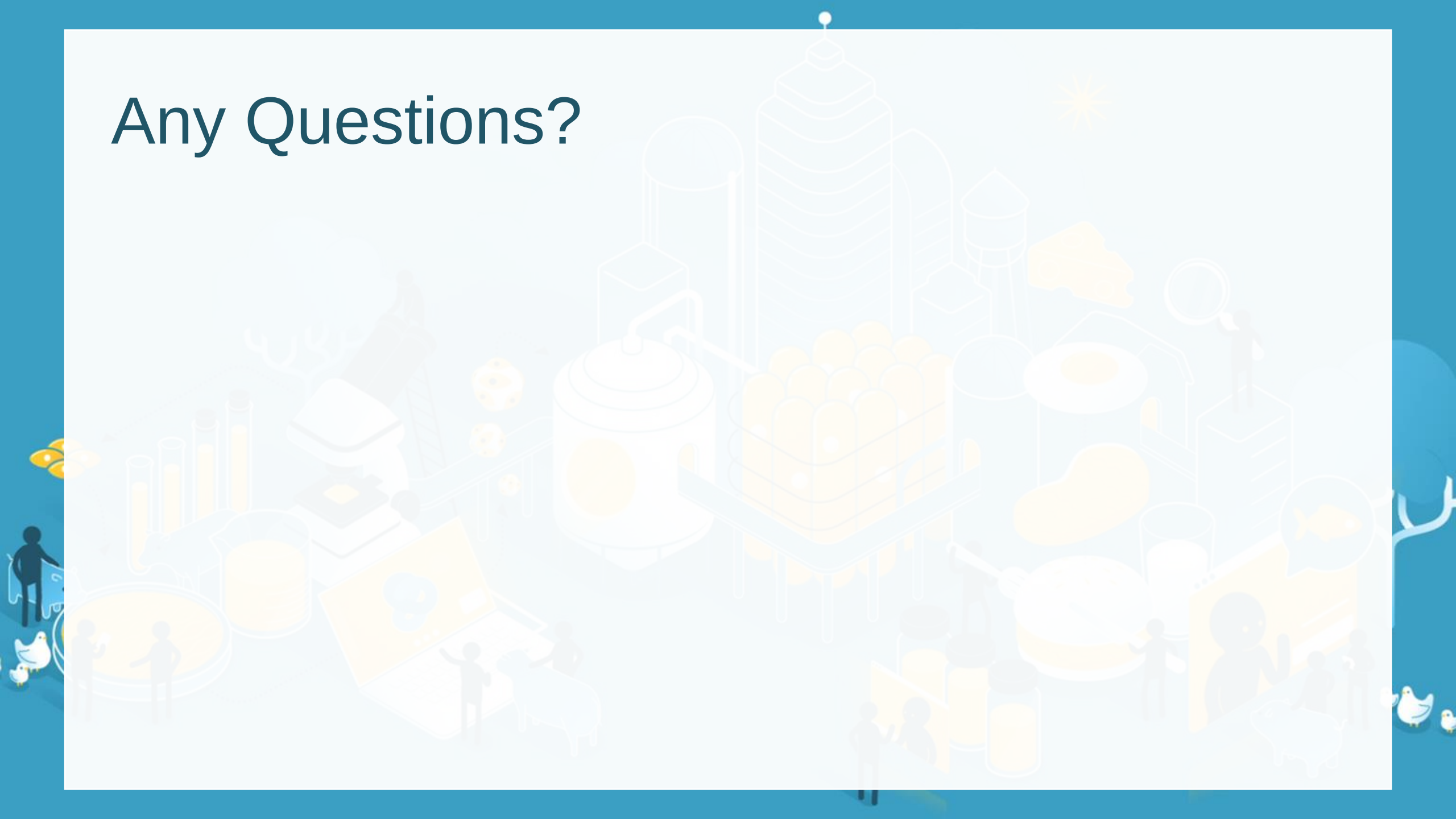
How Do We Passage?

- Cell scraping
- Enzymatic
  - **Trypsin EDTA**
  - Accutase/accumax
- Non-enzymatic solutions
  - Chelation





Any Questions?



Today's Plan

- Passage / sub-culture cells for cryopreservation in week 4
- To seed cells for myogenic and adipogenic differentiation in week 4
  - Seed a 12-well plate
    - 6 wells of stromal vascular cells (adipogenic precursors)
    - 4 wells of satellite cells (myogenic precursors)

    (Will need to coordinate cell hand-offs between groups)

Today's Protocol (Part I)

- Rinse with DPBS to remove FBS
- Add 0.25% trypsin-EDTA
- Incubate at 37°C for ~5 minutes
- Confirm detachment on microscope
- Neutralize trypsin with growth media, move solution to tube
- Spin down the cells at 300 g for 5 mins to pellet cells
- Resuspend in 5 mL GM then count

Today's Protocol (Part II)

- Seed cells: either for expansion or for adipogenicity test
- For expansion, seed cells at 1,000 (muscle) or 2,000 (fat) cells/cm^2 in flasks
- For differentiation, seed cells 2,000 (muscle) or 4000 (fat) cells/cm^2 in a 12-well plate
  - 6 wells with stromal vascular cells
  - 4wells with satellite cells

Next week

- Cryopreservation
- Adipogenic media screen
- Myogenicity test