

- **Announcements**
- **Pre-lab Lecture**
  - ❖ Tissue culture basics
  - ❖ Tissue engineering (TE) toolkit
  - ❖ Mod 3 overview
  - ❖ Today in Lab (M3D1)

# Announcements

- Module 2 report due Wed 4/23 at 5 pm
  - recall: free late day if you get documented writing help
  - methods graded by section instructor, rest by Leona
  - comments about the master spreadsheet
- Next time:
  - arrive in 2 shifts (1 or 3 pm)... quiz before or after
  - anyone available on Tuesday? N : (
- Office hours:
  - cancelled Monday
  - fill out Doodle for additional times this and next week
- Introducing... Reggie, your TA for Module 3

same cells/DNA

intact 1  
intact 2  
cut 1  
cut 2

arbitrarily  
but that's fine  
small error

→ singlet vs. duplicate  
vs. class-wide denominator  
↳ risky

T  
↑

SAT, T ?  
↑

# Mod 2 intro FNT

(personal communication)  
(unpublished work)

- Tone: academic vs. pop sci
- Structure: topic sentences and smooth transitions
- Content:

– Big picture: don't stay too broad, too long  
establish motivation, not list of facts

– Zoom in: background: NHEJ pathway cell lines, (assay systems for repair)

} (DSBs)

precedent doing some citation

– Specific experiment: assay system  
+ question why topology?

- Don't forget the hypothesis!

filoviruses are single-stranded, negative sense RNA viruses that cause severe hemorrhagic fever in humans and nonhuman primates. (1) The family Filoviridae includes genera Ebolavirus and Marburgvirus and a proposed genus of Cuevavirus. (2) The genus Ebolavirus contains five species, which are Zaire Ebola virus (EBOV), Sudan virus (SUDV), Tai Forest virus (TAFV), Reston virus (RESTV), and Bundibugyo virus (BDBV), ... (3) Among the five species of Ebolavirus, RESTV does not cause disease in humans, although it is pathogenic in nonhuman primates. (4, 5) However, RESTV was recently isolated from a swine population in the Philippines, whose animal handlers were seropositive suggesting the potential zoonotic nature of filoviruses. (5, 6) Together, these observations highlight the potential public health risk posed by filoviruses and the need to develop innovative countermeasures.

<http://www.ncbi.nlm.nih.gov/pubmed/24067006>

## Sensationalistic

At first, people infected with the Ebola virus appear to have the flu—fever, chills, muscle aches. Then the bleeding begins. As the virus hijacks cells throughout the body to make copies of itself, it overwhelms and damages the liver, lungs, spleen and blood vessels. Within days organs begin to fail and many patients fall into a coma. Some outbreaks, primarily in Central and West Africa, have killed up to 90 percent of infected individuals.

<http://www.scientificamerican.com/article/could-rna-drugs-defeat-ebola-virus/>

Lung cancer is the leading cause of cancer death in males and the second-leading cause of cancer deaths in females worldwide [1]. In the past decades, lung adenocarcinoma, one histological subtype of non-small cell lung cancer (NSCLC), has become the most common histologic type among all lung cancers diagnosed [2]. Platinum based combination chemotherapy is the standard chemotherapy for NSCLC, and cisplatin is widely used for the treatment of lung cancer [3]. However, individuals respond to chemotherapy differently and the efficacy of cisplatin treatment is often impaired by the emergence of resistance to this drug [4]. Therefore, elucidating the mechanism underlying the development of chemoresistance would promote our understanding of lung cancer progression and treatment failure.

Ma et al., Journal of Experimental & Clinical Cancer Research 2012, 31:99

Physicians have long marveled at the body's ability to heal itself. Over time, breaks, tears, burns and bruises can often disappear sans medical intervention. Less well-understood are the similarly extraordinary repairs that take place on the molecular level, in DNA....Ultraviolet radiation, chemotherapy and other agents can cause lesions in cellular DNA that must be fixed before the cell divides and replicates the mutations, which can lead to cancer, among other problems. Previous work had implicated ATR in the repair of damaged DNA, but exactly which part of that cascade of events the protein is responsible for remained a mystery.

<http://www.scientificamerican.com/article/researchers-identify-prot/>

Courtesy Shannon H.

# ~~Tissue culture (TC) environment~~

Regeneration

What is the "cellular environment" *in vitro*?  $37^{\circ}\text{C}$ ,  $\text{O}_2$

in vitro

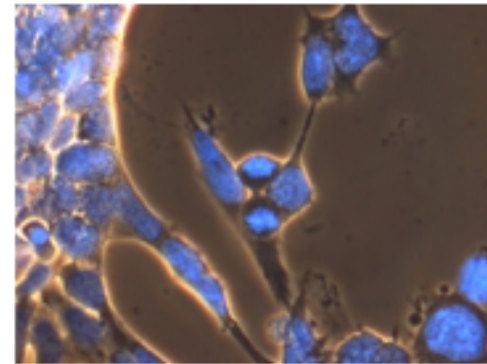
make  
it  
work

in vivo

- extracellular matrix
  - fibronectin, collagen, laminin
  - GAGs, mucin
- cytokines, including GFS
  - many ~~are~~ bind to matrix
  - can be autocrine, paracrine, ...

• nutrient supply

→ blood and lymph flow X not for cartilage



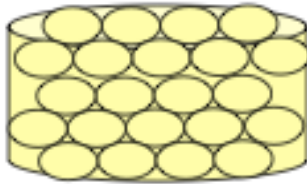
Murine embryonic cells

Image from [http://www.stemcellresources.org/library\\_images.html](http://www.stemcellresources.org/library_images.html)

# Tissue engineering toolkit

**scaffold/matrix**  
→ usually degradable, porous

*alginate (+)*  
*nano vs micro porous*



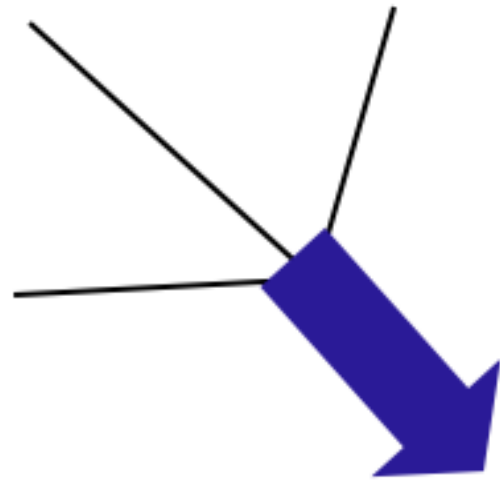
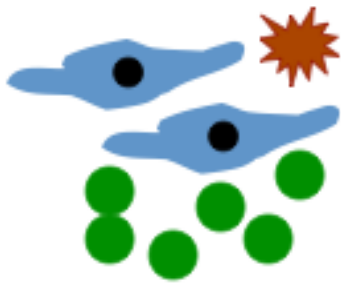
**soluble factors**  
→ made by cells or synthetic  
→ various release profiles

*most freedom*

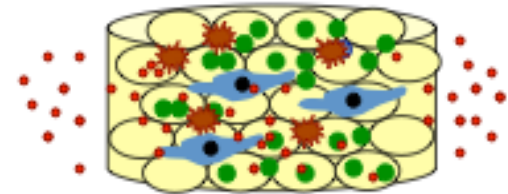


**cells**  
→ precursors and/or differentiated  
→ usually autologous

*CDR or MSCs*



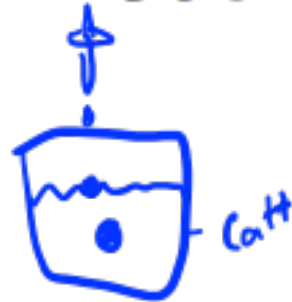
*in vitro*  
**integrated implantable or injectable device**



# First half module overview: lab

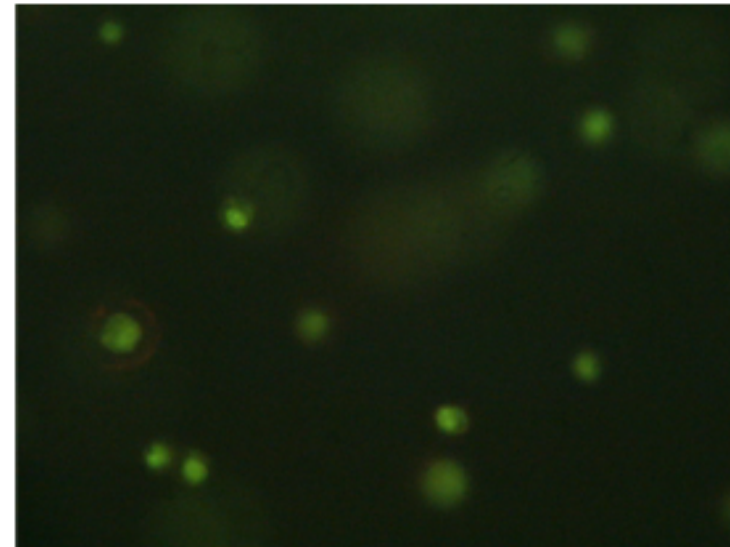
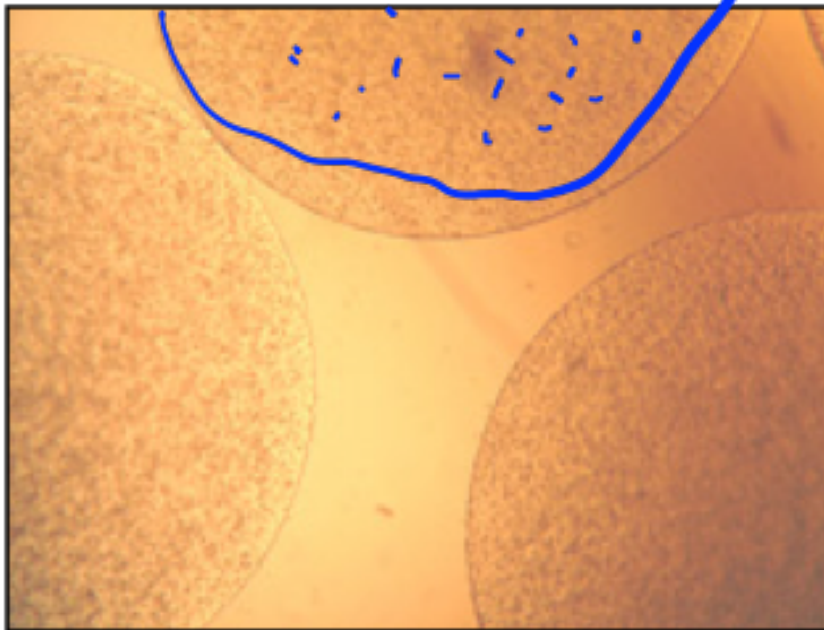
Day 1: design

Day 2: seed cultures



Day 3: viability assay

(timing)



# Today in Lab: research + design

by default use intermediate % of Zish low  
62 ish median

- Skim  $\geq 4$  out of 10 articles (and others!)
  - Read abstract
  - Skim methods: typical abstract % cell  $\rho$ , etc.
  - Skim results/discussion: summarize in 1-2 sentences
  - Goal: get desired info (vs. deep reading)  $\sim 3-5$   
in  $M1+M2$

- Make your own plan - collaborate?
  - Vary one parameter: simple or sophisticated
  - Check cell availability with teaching faculty
  - Request unique materials/equipment needed

- Goal: choose an expt'l Q (and a hypothesis) and conceive of your design  $\rightarrow$  hand in  $\vdots$