Mammalian Cell Culture
Week 3

BIOE202 Spring 2016
Dr. Karin Jensen
Questions from last lab/lecture?
Visualizing cells

In BIOE202 we have used:

- Trypan blue stain
- Phase contrast microscopy

http://www.plantmanagementnetwork.org/pub/php/research/2013/viability/
Historical perspective

Paul Ehrlich
(Nobel Prize 1908)

Nobelprize.org

http://1aled.fotomaps.ru/textile-dyeing.php
Historical perspective

Paul Ehrlich
(Nobel Prize 1908)

http://www.paul-ehrlich.de/Research/research.htm
“It has occurred to me that...it should be possible to find artificial substances which are really and specifically curative for certain diseases...”
Historical perspective

Trypan red

Trypanosome parasites

Compound 606-Salvarsan
Medical uses of tissue staining

• H&E stain
  – Hematoxylin-blue stain that is basophilic/positive (binds acidic/negatively charged components, ex. DNA/RNA)
  – Eosin-red stain that is acidic/negative (binds acidophilic/positive, ex. Most cytoplasmic proteins)
• Primary diagnostic technique for pathologists
• 2.5-3 million slides per day!

http://www.jcancer.org/v03p0058.htm
Fixing cells

Fixation is a critical step before cell staining

Why?

• Preserve cells and tissue structure
• Prevent autolysis (self digest by enzymes)
• Prevent decomposition
How do we fix cells?

- Organic solvents (alcohols or acetone)
  - Remove lipids
  - Dehydrate tissue
  - Denature and precipitate proteins

- Chemical crosslinkers (formaldehyde, glutaraldehyde)
  - Covalent chemical bonds are formed between proteins and surroundings

Formaldehyde
Formaldehyde terminology

• Formaldehyde and formalin
  – Difference in composition (% formaldehyde)
  – Stock formaldehyde is 37-40% formaldehyde
  – Formalin % is diluted 37-40% formaldehyde
    • Ex. 10% formalin is 3.7-4% formaldehyde

• Neutral buffered formalin
  – Formaldehyde in PBS
Formaldehyde Safety

• Formaldehyde is an extremely hazardous material
• Safety glasses and nitrile gloves must be worn at all times
• Formaldehyde should only be used in the fume hood
• The SDS is in the ELN
Formaldehyde Waste

• All waste must be collected
Oil Red O (ORO) stain for lipids

Fat soluble dye

Why do we need ORO?

• Lipids are chemically unreactive
• Lipids are hard to preserve during tissue processing

http://www.niddk.nih.gov/about-niddk/staff-directory/intramural/elisabetta-mueller/Pages/research-images.aspx
Applications for ORO

Medical Diagnostics
• Bone fractures or crush injuries
• Tumors (lipomas and liposarcomas)

Research
• Disease studies
• Fat metabolism

Forensics
• Fingerprints

Pyrotechnics
Other ways to visualize cells

- Immunofluorescence
- RNA FISH
- Fluorescent tagged proteins
- Fluorescently conjugated toxins (Module 5)
- All these techniques can be quantified by the methods you are learning in ImageJ software!

Mcb.berkeley.edu

Janes et al. Nat Methods. 2010
Cell lysis: Detection of proteins

- 250,000-1 million different proteins in a cell!
- Changes in protein levels and modifications are important in biological processes
- Important in many research studies and for clinical diagnoses

3T3-L1 cells
Cell lysis experiment

• We will analyze a protein change that occurs in response to a high dose of serum.

• External factors (like serum) can signal the cell to change protein and transcription factors.
Cell lysis experiment

• Target protein: extracellular regulated kinase 1/2 (ERK1/2)
• Phosphorylated when activated
• Key signaling protein in a common growth pathway
• This pathway is commonly deregulated in cancer cells
• We will quantify our lysates in Module 6 and analyze changes in phosphorylated ERK1/2 protein in Module 7

Pratilas C A, Solit D B Clin Cancer Res 2010;16:3329-3334
Module 4 Week 3 Lab Overview

Main Lab

Differentiated 3T3-L1s

6cm → Fix → ORO → Image and Analysis

Return Lab

T25 growth media

A → F growth media

cryovial in liquid nitrogen

37 deg C → 3T3-L1s
Final Project

Study the affect of a variable of your choice on 3T3-L1 cells

Should have clear motivation of why we would want to study this (what are the applications? Tissue engineering, cancer?)

Your lab team will develop a complete experimental plan with appropriate controls and replicates to complete the study
Final Project

Examples
Response to varying media components (ex. High vs. low serum)
Response to varying environment (ex. Varying CO$_2$ or O$_2$)
Response to a drug/inhibitor
Adhesion on varying substrates (ex. Collagen)
Affect of a variable on differentiation (passage number, media components, etc.)
Final Project

What measurements can you take?
Cell growth (image analysis)
Morphology (image analysis and/or fluorescence microscopy)
Growth signaling (Western blot)
Final Project

- Pre-proposal due March 16\textsuperscript{th} at 2pm
- Full proposal due April 1\textsuperscript{st} at 5pm
- Project begins week of April 11\textsuperscript{th}
- Final report (1-page BMES style abstract) will be due May 13\textsuperscript{th} at 5pm

The pre-proposal and proposal will be group submissions and will together count as one assignment.

More details will be posted on the course website.
Reminder: Lab Practical

• Week of March 14th during your normal lab time
• The protocol you will be given will be posted on the course website-no surprises!
• Practice, practice, practice!
• Sign up for a time next week
• Questions?
Lab Practical

• **15% of your final grade**
• **Week of March 14th**
• **Trypsinizing, counting cells, and plating cells and answering questions in the presence of the TA/instructor**
• **During your normal lab time (Main or Return Lab)**
• **You will be given a protocol for trypsinization ahead of time and can use this during the exam**
• **Practice, practice, practice!**
• **Be sure to alternate lab members in the BSC in the upcoming weeks**
Lab Practical

• Skills and concepts we will be checking:
  – Aseptic technique (handling cells, bottles, pipettes...)
  – Preparing BSC and reagents
  – Viewing cells with the microscope
  – Trypsinizing cells
  – Preparation of hemocytometer
  – Counting cells with hemocytometer
  – Calculating cells needed to plate
  – Plating cells
  – Proper disposal of wastes
  – Cleaning BSC
Lab practical details

The protocol that you may use is posted on the course website-please review! No surprises. You will take the practical with Dr. Jensen or one of the TAs.

You will be allowed 45 minutes.

Grade: technique, 6 questions, cells plated correctly

You may **not** ask questions during the practical-we can’t help you!
Schedule and logistics

- Lab next week: Module 4 Week 3
- Lecture next week: Fluorescence Microscopy (Module 5)
- Module 4 assignment is due March 11\textsuperscript{th} at 5pm
- ImageJ guidelines are posted on the course website
Informal Early Feedback

• Please provide me with feedback to improve your learning in BIOE202.
• There are two sides.
• No names please!
• Thank you very much for your comments.