# Viability and Proliferation of Human Dermal Fibroblasts in Culture Conditions

YYY

BIOE 342

# Objectives

- To quantify the relationship between MTT dye absorbance and viability of human dermal fibroblast (HDF) cells
- To observe the effect of ethanol on HDF cell viability
- To determine the relationship between HDF cell proliferation and serum concentration

# MTT Assay Methods

- Cells were diluted from 50,000 cells/mL to 1:1.5, 1:2, 1:3, 1:6, and 1:12 dilutions
- 0.5 mL of cell dilutions were seeded into 12 test wells (2 control wells with complete media) and incubated for 2 days
  - 0.75 µL of MTT dye was added to 7 wells and incubated for 2 hours
  - Remaining 7 wells were used to determine cell count using Coulter Counter
- Absorbance of MTT dye was recorded with spectrophotometer at 570 nm

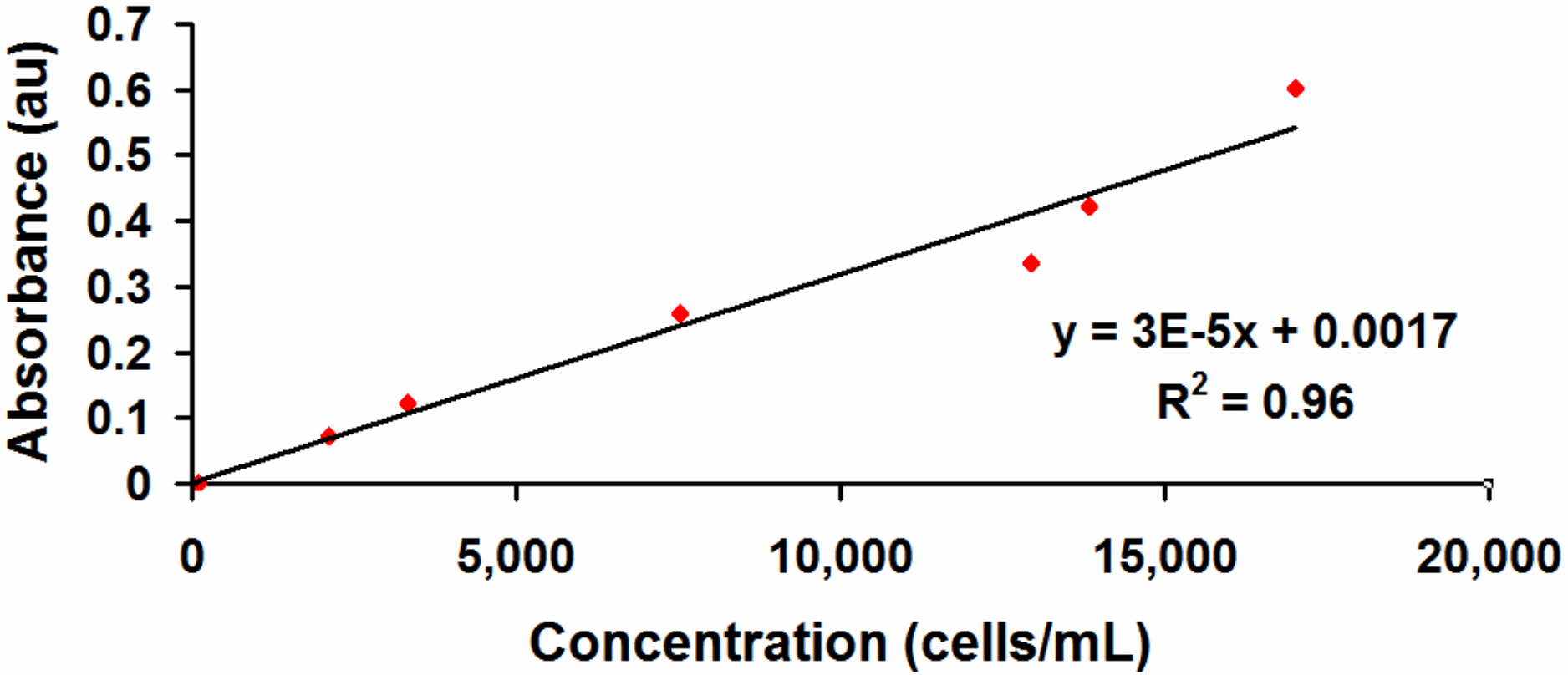
# Live/Dead Fluorescence Assay Methods

- Cells were suspended in 10mL DMEM and diluted 1:3 in complete media
- 1.0 mL of cell dilution was seeded in 9 test wells; incubated for 2 days
- Cell dilutions were treated with 3 different conditions and incubated at room temp. for 30 min.
  - Condition A: 250 µL Phosphate-Buffered Saline (PBS), 100 µL dye
  - Condition B: 250 µL ethanol (EtOH), 100 µL dye
  - Condition C: 2 drops EtOH, 250 µL PBS, 100 µL dye
- Observations under light and fluorescent microscopes were recorded

# Cell Proliferation Assay Methods

- Cells were diluted to 5,000 cells/mL and seeded into 33 total wells in 1%, 5%, or 10% serum conditions
- Cell number was determined with the Coulter Counter after 4 hours, 2 days, 5 days, and 7 days
- DMEM was replenished as needed

# The Linear Relationship Between MTT Dye Absorbance and Cell Concentration



# Absorbance-Concentration Relationship Allows Cell Concentration to be Determined

- Yellow MTT dye is reduced to purple product by metabolizing cells; only viable cells undergo metabolism
- The spectrophotometer measures the absence of purple dye
- Cell viability is directly proportional to absorbance
- Cell concentrations can be determined from the calculated Absorbance-Concentration relationship

# Fluorescent Microscope Observations: Cell Death in the Presence of Ethanol

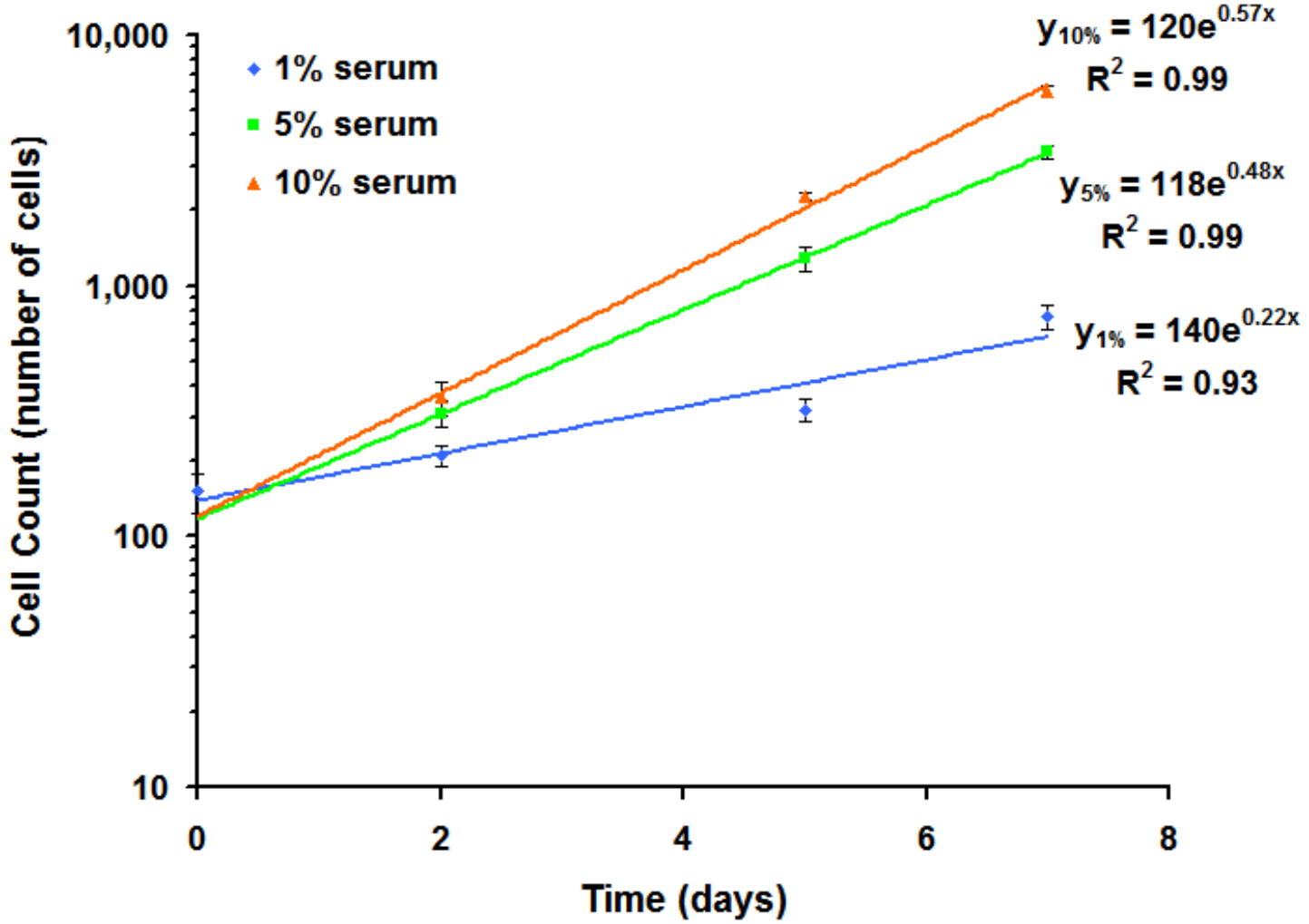
| Condition | Color, Pattern, Morphology |
|---|---|
| 250 µL PBS<br>100 µL dye | 95% of cells stained green; 5% of cell nuclei stained red; uniform cell pattern; majority of cells elongated |
| 250 µL EtOH<br>100 µL dye | ~100% of nuclei stained red; uniform cell pattern; outline of cells appear elongated |
| 2 drops EtOH<br>250 µL PBS<br>100 µL dye | 75% of cells stained green; 25% of cell nuclei stained red; less uniform cell pattern; majority of cells elongated |

- viable cells stain green
- dead nuclei stain red → EtOH decreases cell viability

# Comparison & Contrast Between Cell Viability Assay Results

- MTT and Live/Dead Assay results both show the relationship between cell viability and dye absorbance
- MTT Assay results quantitatively define the relationship between cell viability and MTT dye absorbance
- Live/Dead Assay results qualitatively define the relationship between cell viability and presence of ethanol

# Cell Count Increases Exponentially Over Time



# The Effect of Serum Concentration on Cell Proliferation

- Cell counts at Day 7 for 1%, 5%, and 10% serum are significantly different: ANOVA p-value < 0.001
- Cell doubling time decreases with increasing serum concentrations:
  - 1% serum: 3.59 days
  - 5% serum: 1.45 days
  - 10% serum: 1.15 days

→ Cell proliferation increases with increasing serum concentration

# Conclusions

- There is a linear relationship between MTT dye absorbance and cell viability
- The presence of ethanol decreases cell viability
- Cell proliferation increases with increasing serum concentration