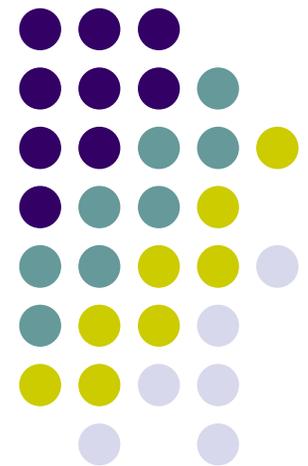


Human Dermal Fibroblast (HDF) Cell Viability and Proliferation In Vitro

YYY
BIOS 320



Objectives



- To examine the relationship between absorbance and HDF cell concentration using a metabolic assay
- To determine the effects of serum concentration on HDF cell cycle and proliferation

Measuring Cell Viability



- MTT Viability Test

- Cells were seeded on two 24-well plates at the following six concentrations diluted from a 50,000 cells/mL stock- 1:1, 1:1.5, 1:2, 1:3, 1:6, and 1:12
- Both plates were incubated for 48 hours
 - Cells on one plate were trypsinized and concentration was measured using a Coulter Counter
 - Cells on second plate were treated with MTT dye and absorbance was measured at 570 nm using a spectrophotometer

Measuring Fraction of Cells in S-phase of Mitosis



- **Anti-PCNA Assay**

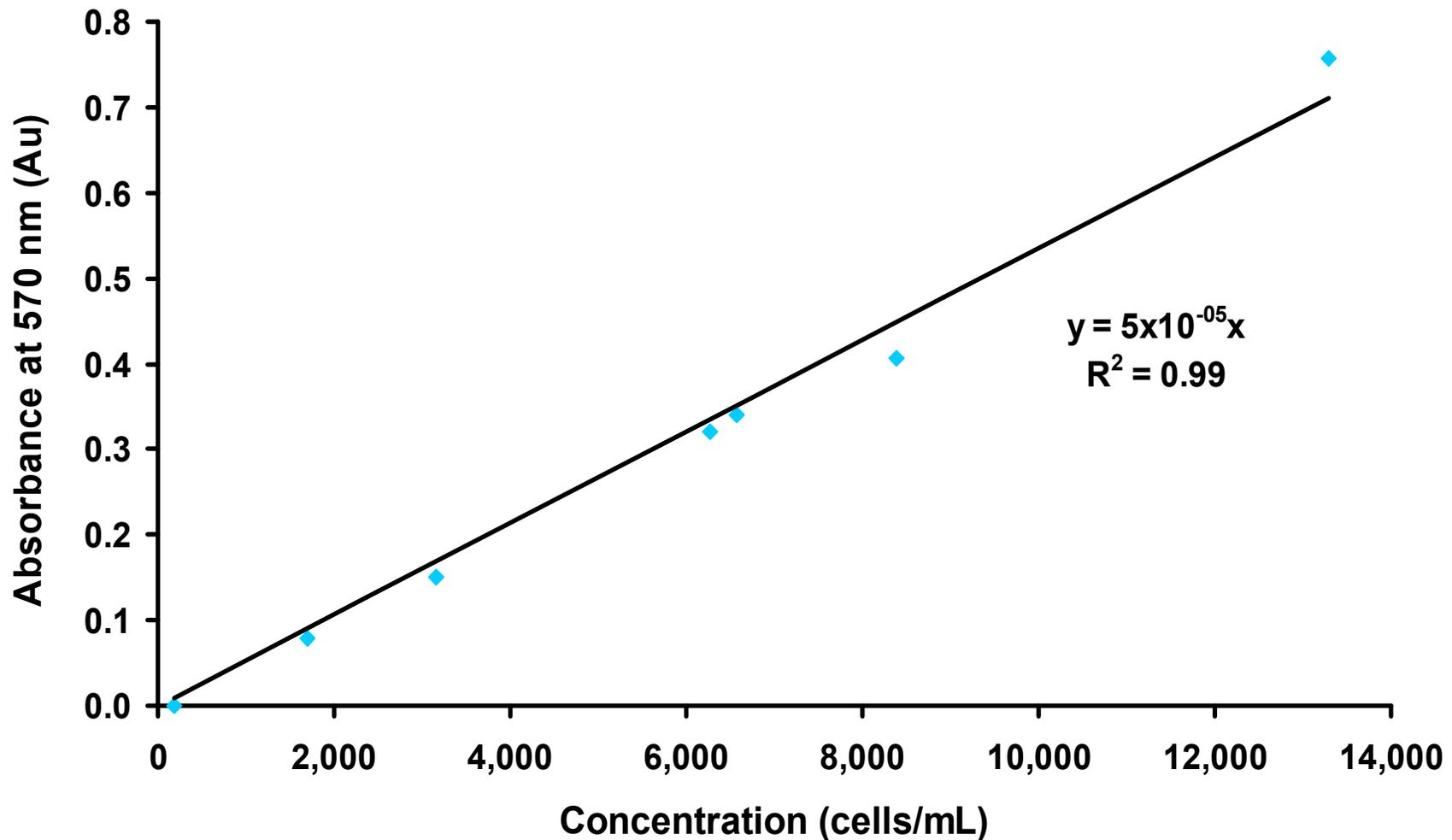
- Proliferating Cell Nuclear Antigen (PCNA) is a protein found in nuclei of cells in S-phase
- Cells seeded at 20,000 cells/mL in DMEM and 1%, 5%, or 10% fetal bovine serum (FBS) and incubated for 48 hours
- Cells treated with primary antibody to PCNA, secondary antibody with horse radish peroxidase (HRP) label, and AEC chromagen and hematoxylin stains
- Cells observed using light microscope to determine percentage of cells in S-phase

Measuring Cell Proliferation



- Cells were seeded at 5,000 cells/mL in DMEM and 1%, 5%, or 10% FBS
- Cells were trypsinized and concentration was measured using a Coulter Counter on Day 0, 2, 5, and 7
- DMEM replenished as needed for wells that were not yet counted

Absorbance of MTT is Linearly Related to Cell Concentration



MTT Assay Reveals Concentration of Viable Cells



- MTT dye is reduced to purple product by metabolizing cells only
- Viable cells have an active metabolism
- Linear relationship between absorbance of dye and concentration allows for concentration of viable cells to be approximated

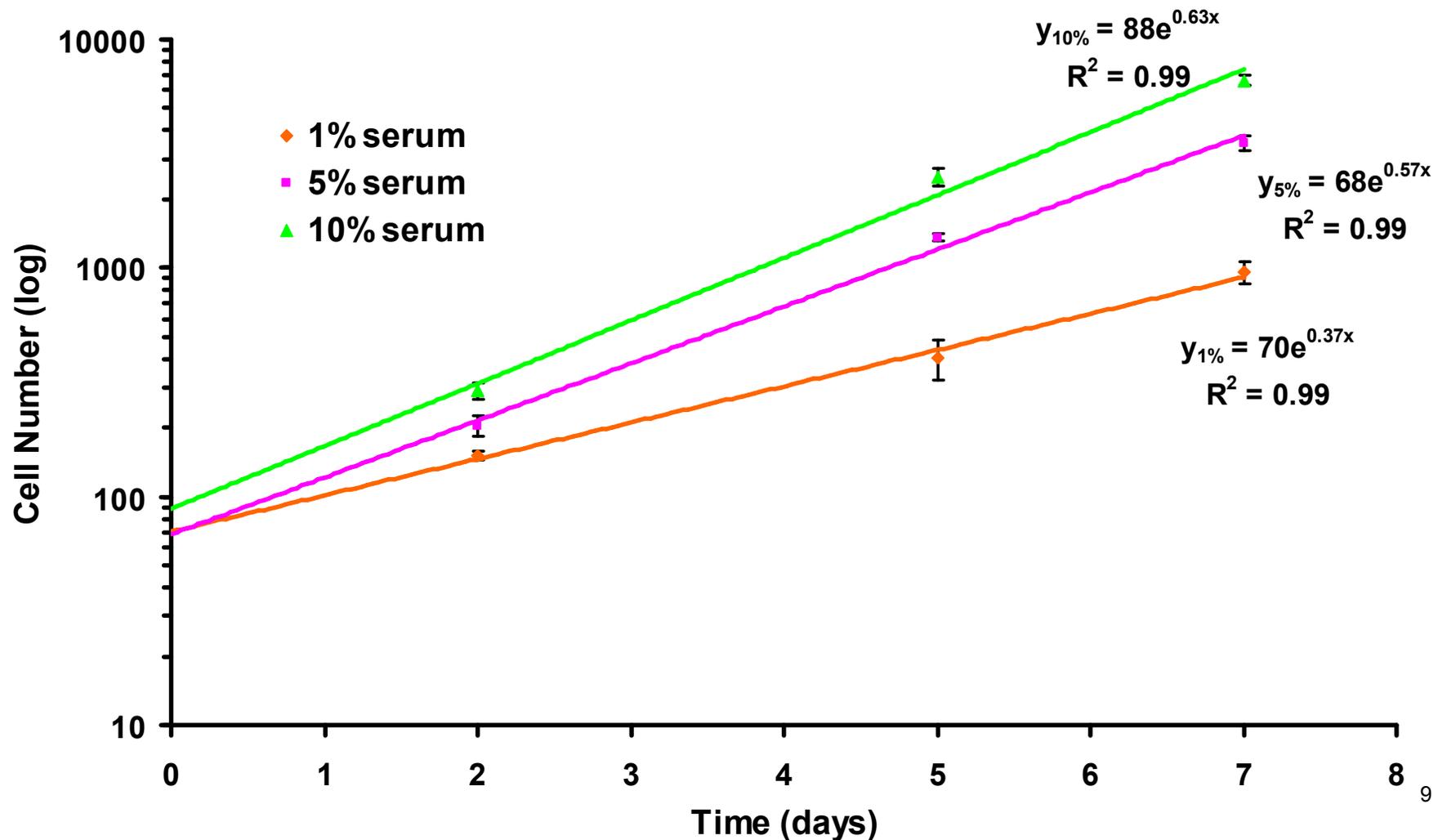
Anti-PCNA Assay Shows Effect of Serum Concentration on Cell Cycle



Conditions	% Nuclei Stained Red
1% FBS	50
5% FBS	70
10% FBS	90

- HRP and AEC chromagen stain nuclei of cells in S-phase red
- More cells are in S-phase at higher serum concentrations

Cell Growth is Exponential in All Tested Serum Concentrations





Effects of Serum Concentration on Cell Proliferation

- Number of cells in 1%, 5%, and 10% serum at Day 7 is significantly different (ANOVA, $p < 0.001$)
- Doubling time decreases with increasing serum concentration
 - 1%: 1.9 days
 - 5%: 1.1 days
 - 10%: 0.98 days
- Cells proliferate faster in higher serum concentrations resulting in more cells

Comparison of Anti-PCNA and Cell Proliferation Assays



- Both assays observe the effect of serum concentration on cell activity
 - Anti-PCNA assay qualitatively examines the effect on fraction of cells in S-phase of mitosis
 - Cell proliferation assay quantitatively reports the effect on cell concentration over time
- In combination, the assays demonstrate that higher serum concentration results in more cell division
 - Greater numbers of cells in mitosis corresponds to more cell proliferation



Conclusions

- A linear relationship exists between absorbance and HDF cell concentration, allowing for estimates of number of viable cells
- Higher serum concentration gives rise to larger number of HDF cells in S-phase and causes more cells to pass through cell cycle, resulting in presence of more cells over time