



# HDF Survival and Function in Vitro

2/13/08



# Objectives

- Use MTT dye to determine relationship between absorbance and HDF cell concentration
- Observe effects of media conditions on the cell cycle and proliferation
- Quantify the growth and replication of HDF cells in different media conditions



# Measuring Absorbance of MTT dye at different Cell Concentrations

- Seeded cells on 2 plates, each with cells at a range of concentrations from 0 to 50,000 cells/mL, incubated 48 hours
- Measured concentration of cells from first plate using coulter counter
- Second plate was treated with MTT dye
  - MTT dye changes from yellow to purple when metabolized by viable cells
- Absorbance of metabolized dye was measured with spectrophotometer



# Determining Fraction of Cells in S-phase using Anti-PCNA

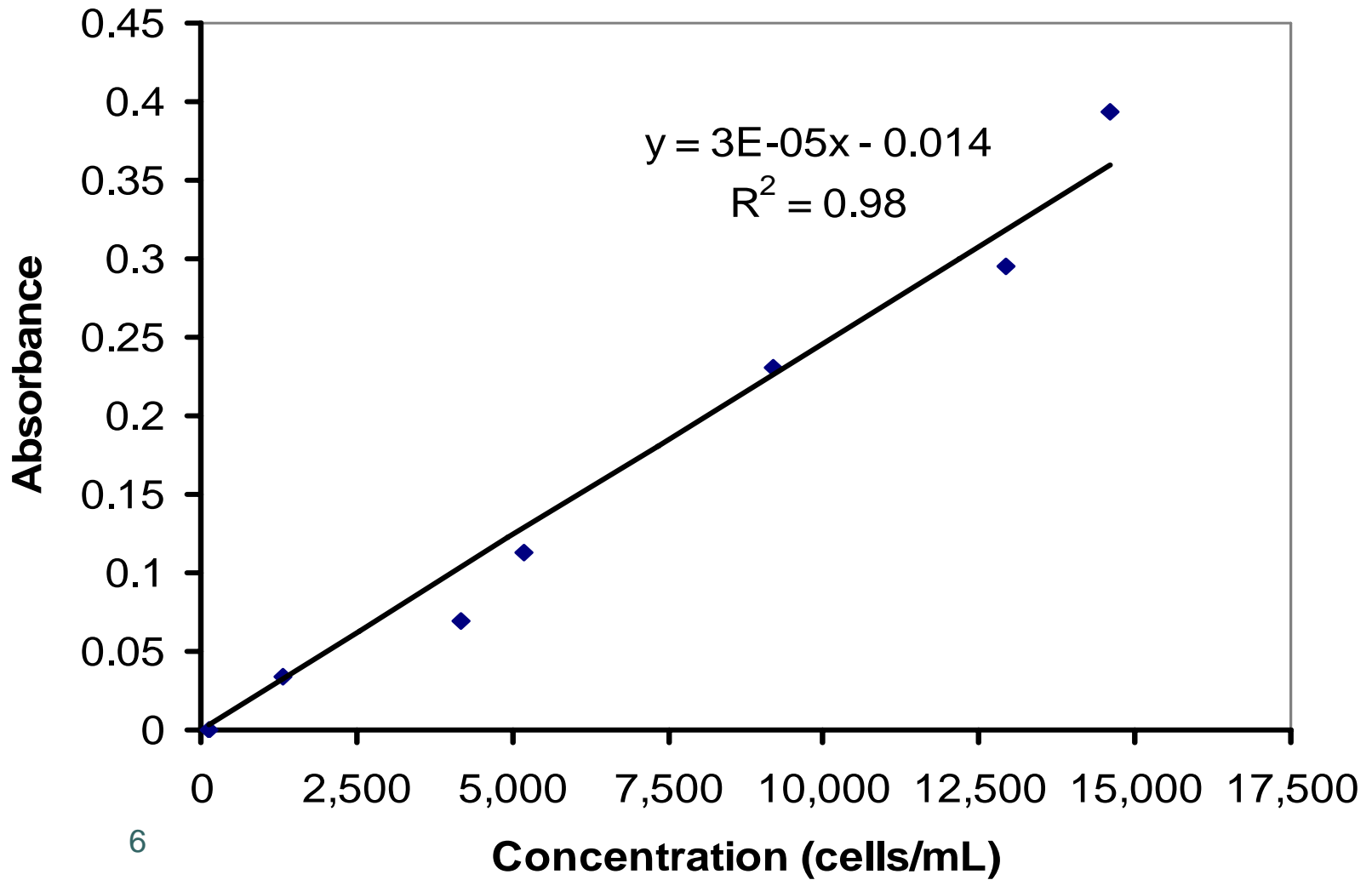
- Same concentration of cells was seeded into wells with media containing 1%, 5%, or 10% FBS as well as control wells, and incubated 48 hours
- Cells were treated with 1° antibody to PCNA and 2° antibody with red color tag
  - PCNA is an S-phase protein found in nucleus
- Fluorescent microscope was used to determine percentage of cells with red nuclei (in S-phase, dividing)



# Measuring Proliferation of Cells in Varying Media Conditions

- Cells were seeded at same concentration in media containing 1%, 5%, or 10% FBS
- Coulter counter was used to measure cell concentration for each media condition at days 0, 2, 5, and 7

A Linear Relationship Exists Between Absorbance of MTT and Concentration of Viable Cells





# MTT Assay Indicates Cell Viability

- Absorbance of metabolized MTT dye is directly related to cell concentration
- Metabolizing cells are viable cells
  - Means that MTT assay provides information about both concentration and viability



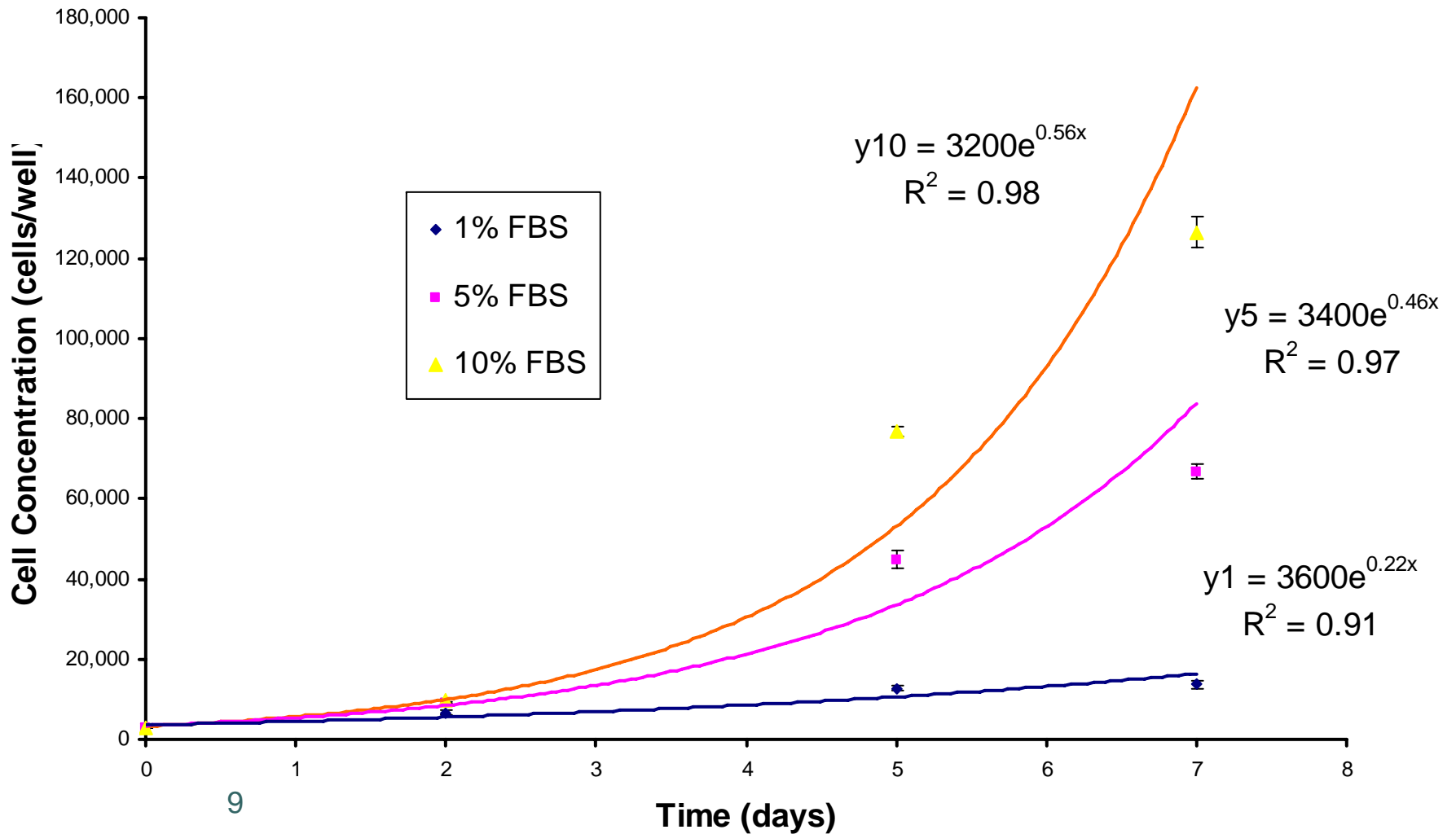
# Anti-PCNA Staining Reveals Fraction of Dividing Cells

Media Condition	% Red Nuclei
control 1 + primary antibody	0
control 2 + secondary antibody	20
control 3	0
1% FBS	40
5% FBS	60
10% FBS	80

- Nuclei stained red are in S-phase and are actively growing/dividing
- Higher FBS content in media correlates with increased growth and proliferation



# HDF Cells Demonstrate Exponential Growth in Varying Serum Concentrations





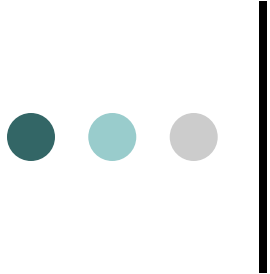
# Cells Grow Exponentially at Different Rates

- Cells proliferate faster in higher serum (FBS) content
  - By days 5 and 7, there is a statistical difference between cell concentration in each %FBS ( $p < 0.01$ )
  - Growth rates expressed as cell doubling time
    - 1% FBS: 3.6 days
    - 5% FBS: 1.5 days
    - 10% FBS: 1.2 days



# Anti-PCNA and Proliferation Assay Show Similar Effects of Serum Content

- Anti-PCNA assay shows that %FBS affects fraction of cells in S-phase, while Proliferation assay quantifies growth over time in different %FBS
- Anti-PCNA results are qualitative while proliferation assay shows quantitative change over time
- Must assume that cells in S-phase are growing and proliferating to make connection between assays



# Viability and Proliferation Assay Conclusions

- Relationship between cell concentration and absorption of metabolized MTT dye (representing viable cells) is linear
- Increasing serum concentration up to 10% FBS correlates with:
  - Increase in fraction of cells in S-phase (cells growing/proliferating)
  - Increased proliferation rate