HDF Proliferation and Viability

YYY

Objectives

- Study quantifiable effects of 1%, 5%, 10% FBS (Fetal Bovine Serum) on HDF (Human Dermal Fibroblasts) proliferation
- Find relationship between 1%, 5%, 10% FBS and HDF cell cycle
  - Anti-PCNA Staining
- Construct spectrophotometer standard curve for HDF cells
  - MTT Viability Test

MTT Viability Test Methods

- Vary HDF P4 dilutions of 50,000 cells/mL
  - 1:1, 1:1.5, 1:2, 1:3, 1:6, 1:12; control
  - Control: no cells
- Coulter Counter to determine cell concentrations
- MTT dye added to identify cells in S-phase
- Measure absorbance at 570nm for each dilution

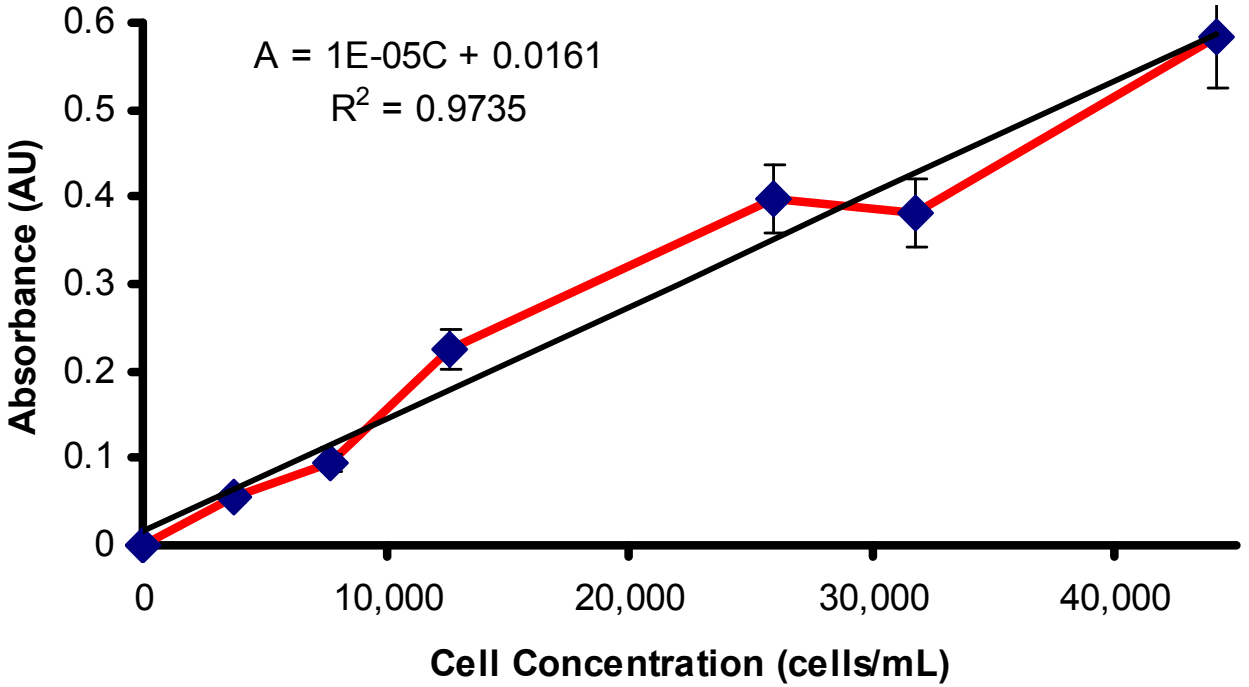
Anti-PCNA Staining Methods

- Vary FBS concentrations with HDF P6 cells
  - 1%, 5%, 10% FBS concentrations in DMEM
  - Control 1: 10% FBS and primary antibody
  - Control 2: 10% FBS and secondary antibody
  - Control 3: 10% FBS and no antibodies
- Seed 20,000 cells/mL into test conditions
  - 1 well per test condition
- Add primary antibody: Mouse IgG
- Add secondary antibody: HRP
- Count percentage of red-stained nuclei with hemocytometer

Cell Proliferation Assay Methods

- Vary FBS concentrations with HDF P6 cells
  - 1%, 5%, 10% FBS concentrations in DMEM
- Seed cells 5,000 cells/mL on Day 0
- 3 wells per test condition per day
  - Day 0 contains 6 wells
- Determine cell concentrations on Days 0,2,5,7
  - Count cells with Coulter Counter

MTT Test Standard Curve



- Linear relationship between cell concentration and absorbance
- HDF cells

1% FBS Has Least Cell Cycle Activity

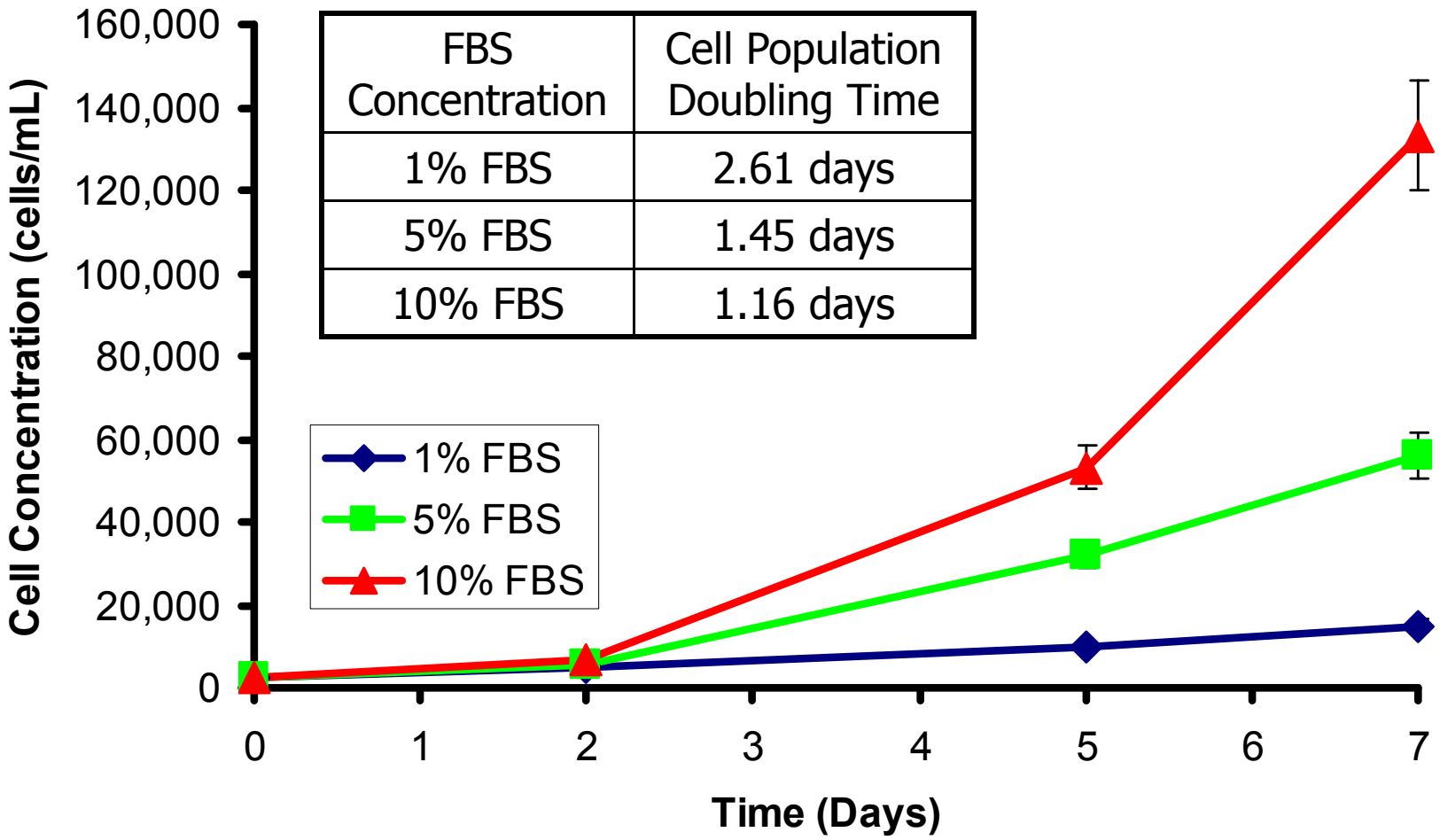
- Anti-PCNA
  - Red-stained nuclei indicates cells in S-phase
  - Blue-stained nuclei indicates cells not in S-phase

FBS Concentration	Cells in S-phase (%) (Stained Red)
1% FBS	53.3
5% FBS	59.3
10% FBS	60.3
Controls	0

- 1% FBS least cells in S-phase
- 10% FBS most cells in S-phase

Cell Proliferation With Different FBS Concentrations

FBS Concentration	Cell Population Doubling Time
1% FBS	2.61 days
5% FBS	1.45 days
10% FBS	1.16 days



Statistical Differences Exist Between Both Day Number and FBS Percentage

- ANOVA tests

Day Number (vary FBS concentration)	Statistically Significant Difference Between Concentrations	P Value
0	No	1
2	Yes	0.0015
5	Yes	$8.29*10^{-6}$
7	Yes	$2.19*10^{-7}$

Percentage FBS (vary Day#)	Statistically Significant Difference Between Concentrations	P Value
1%	Yes	$1.26*10^{-8}$
5%	Yes	$6.03*10^{-13}$
10%	Yes	$4.94*10^{-15}$

- There are statistical differences in the cell concentrations between Days 2, 5, and 7 as well as between 1%, 5%, and 10% FBS

10% FBS Lowest Doubling Time

- Increase seen in cell concentration as FBS increases from 1% to 5% to 10%
  - Increase becomes larger as Day Number increases
- Doubling time is lowest for 10% and highest for 1%

Cell Proliferation and Anti-PCNA

- 1% FBS results in decreased cell cycle activity and cell proliferation
- 5% and 10% FBS results in similar cell concentrations and cell cycle activity after 2 days of incubation

Discussion

- 1% FBS does not allow efficient cell population growth
  - Limited amount of nutrients
  - Limited number of cells divide
  - Do not use 1% FBS to growing cell populations
- Cells in 10% FBS proliferate fastest among 1%, 5%, and 10%
  - Lowest doubling time
  - Sufficient amount of nutrients for cells
- MTT Standard Curve shows linear behavior between absorbance and cell concentration
  - Can use standard curve for future samples of HDF cells with unknown concentrations