Viability and Proliferation in Human Dermal Fibroblasts (HDF)

BIOE 342

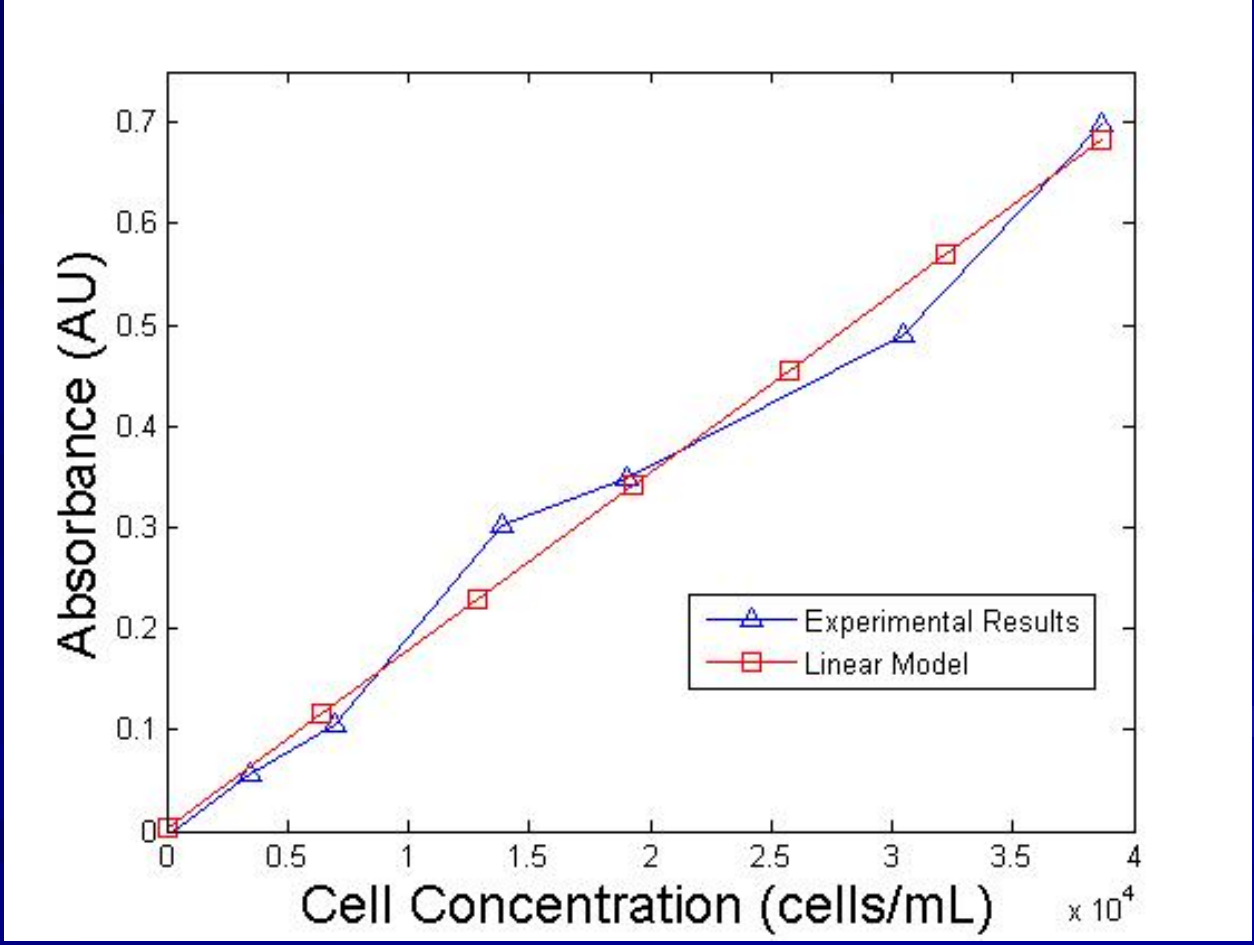
Objectives

- The MTT Viability Test seeks to determine the relationship between cell concentration and absorbance after treatment with MTT dye.
- Proliferation assays seek to determine the effects of different serum concentrations on the following:
  - Anti-PCNA Staining: DNA synthesis
  - Cell Proliferation Assay: cell proliferation

MTT Viability Test Methods

- Stock solution of 50,000 cells/mL was prepared.
- 1:1.5, 1:2, 1:3, 1:6, and 1:12 dilutions, as well as stock (no dilution) and control (no cells), were seeded at half a mL in 2 plates each.
- Cell concentration of 1 plate per condition was measured using the Coulter Counter.
- 1 plate per condition was treated with MTT dye.
- After MTT dye treatment, absorbance of solution in each plate was tested using the spectrophotometer.

Cell Concentration Causes Linear Increase in Absorbance



- Linear Model: Absorbance = $2.74*10^{-3}+1.75*10^{-5}*$(Cell Concentration), $R^2=.983$

Linear Relationship between Cell Concentration and Absorbance

- Linear relationship predicted by Beer-Lambert Law.
- R^2 value of .983 indicates that linear model based on experimental results is an extremely good fit.
- Thus, MTT Assay can be used to predict cell concentration with known model.

Anti-PCNA Staining Methods

- 1 mL of 20,000 cells/mL solution was seeded in three wells at 1%,5%, and 10% Fetal Bovine Serum (FBS) concentration.
- Cells were treated with anti-PCNA antibody, anti-mouse IgG antibody, AEC solution, and hematoxylin.
- Stained cells were subsequently observed under a light microscope.
- Nuclei stained red indicated presence of PCNA and thus DNA synthesis.

Increased FBS Concentration Increases DNA Synthesis

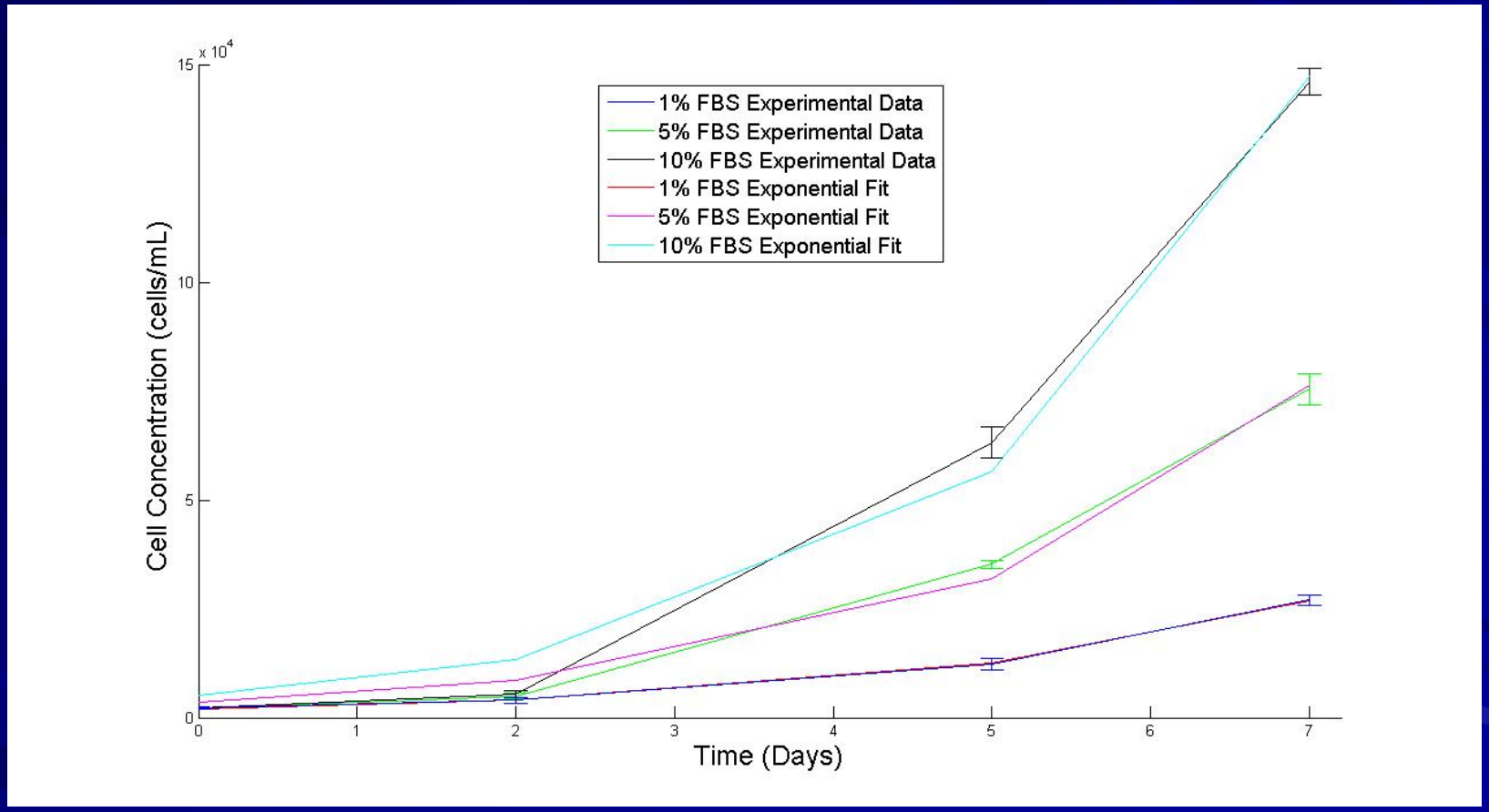
- DNA synthesis is a precursor to proliferation.
- Cells cultured in higher FBS concentration more likely to be replicating DNA
- As this experiment doesn't directly measure proliferation, Cell Proliferation Assay was conducted.

FBS Concentration	Approximate % of cells in DNA Synthesis
1%	40%
5%	60%
10%	85%

Cell Proliferation Assay Methods

- 6 wells were seeded with 1 mL of 5,000 cells/mL at 1% FBS; cell concentration of these wells was determined after 4 hours using the Coulter Counter.
- 9 more wells were seeded with 1mL of 5,000 cells/mL at each 1%, 5%, and 10% FBS concentration.
- On Days 2, 5, and 7, cell concentrations of 3 wells of each 1%, 5%, and 10% FBS were measured using the Coulter Counter.

Proliferation of HDF Promoted by Higher FBS Concentrations



- Error Bars represent +- one standard deviation.

Statistical Analysis of Proliferation at Different FBS Concentrations

- Exponential Fit Models:
  - 1% FBS: $1900*e^{.38*x}$; $R^2=.99$
  - 5% FBS: $3600*e^{.44*x}$; $R^2=.99$
  - 10% FBS: $5200*e^{.48*x}$; $R^2=.99$
- Tukey's HSD F-Values on Final Concentrations:
  - 1% FBS vs. 5% FBS: <.025
  - 5% FBS vs. 10% FBS: <.01
  - 1% FBS vs. 10% FBS: <.005

Comparison of Differing FBS Concentrations on Cell Proliferation

- Exponential models are highly effective fits and show different rates of growth.
- Doubling times:
  - 1% FBS: 1.4 days
  - 5% FBS: 1.6 days
  - 10% FBS: 1.8 days
- Tukey's HSD test results show statistically significant differences between all three pairs of concentration data at Day 7.

Effect of Serum Concentration on HDF Proliferation

- Anti-PCNA Staining shows cells with more FBS present are more likely to be replicating DNA, a precursor to mitosis.
- Cell Proliferation Assay shows cells to grow faster under greater serum concentrations.
- Taken together, these data indicate that greater serum concentration promotes more frequent cell division.

Summary

- MTT Viability Test shows a linear relationship between cell concentration and spectrophotometer absorbance in cells treated with MTT dye.
- Proliferations assays (Anti-PCNA Staining and Cell Proliferation Assay) show that greater FBS concentrations increase cell proliferation.