Characterization of Human Dermal Fibroblast (HDF) Function In Vitro

YYY

Objectives

- To determine the relationship between cell concentration and absorbance at 570 nm.
  - MTT Viability Test
- To assess whether serum concentration can significantly affect HDF cell proliferation.
  - Anti-PCNA Staining and Cell Proliferation Assay

Methods: MTT Viability Test

- Cells were seeded in duplicate plates in DMEM with 10% fetal bovine serum (FBS) and 1% antibiotic, and incubated for 2 days.
  - 50,000 cells/mL (stock). Plated at 1:1, 1:1.5, 1:2, 1:3, 1:6, 1:12 dilutions.
- After 2 days, one plate was trypsinized and Coulter counted while the other was treated with MTT dye.
  - Absorbance at each cell concentration was measured with the Genesys 20 Spectrophotometer.

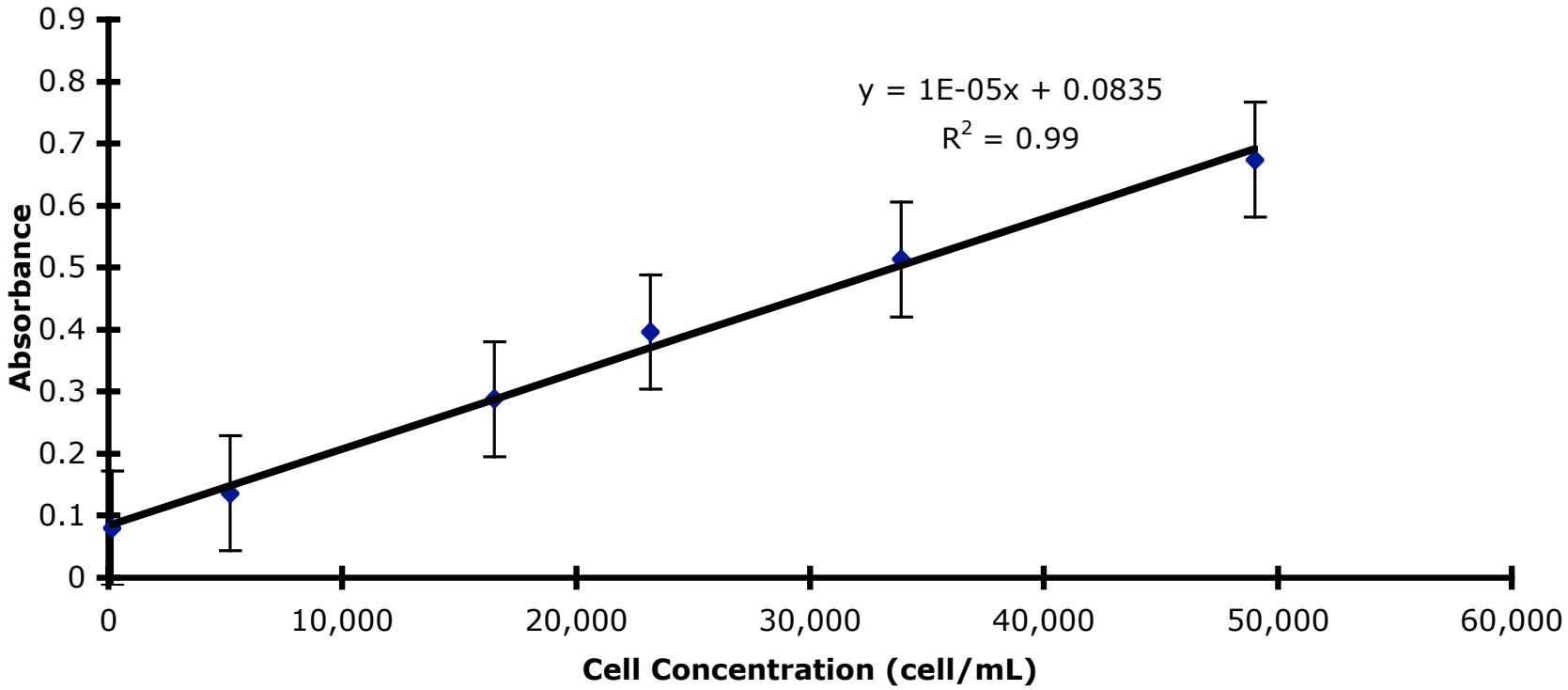
Methods: Anti-PCNA Staining

- Cells were seeded at 20,000 cells/mL in DMEM with 1%, 5%, and 10% FBS and incubated for 2 days.
- Anti-PCNA primary antibody was added, followed by anti-mouse secondary antibody.
  - Cells were subsequently treated with AEC chromagen and hemotoxylin stains.
- Fluorescent microscopy was then used to count and calculate the percentage of cells in S-phase (stained red).

Methods: Cell Proliferation Assay

- Cells were seeded at 5,000 cells/mL in DMEM with 1% antibiotic and 1%, 5%, or 10% FBS.
  - Incubated for 7 days with time points at 4 hours, 2 days, 5 days, and 7 days.
  - At each time point, cells in each serum condition were trypsinized and concentrations were calculated from Coulter counts.

Increasing Cell Concentration Increases Absorbance



Viable HDF cells increase absorbance

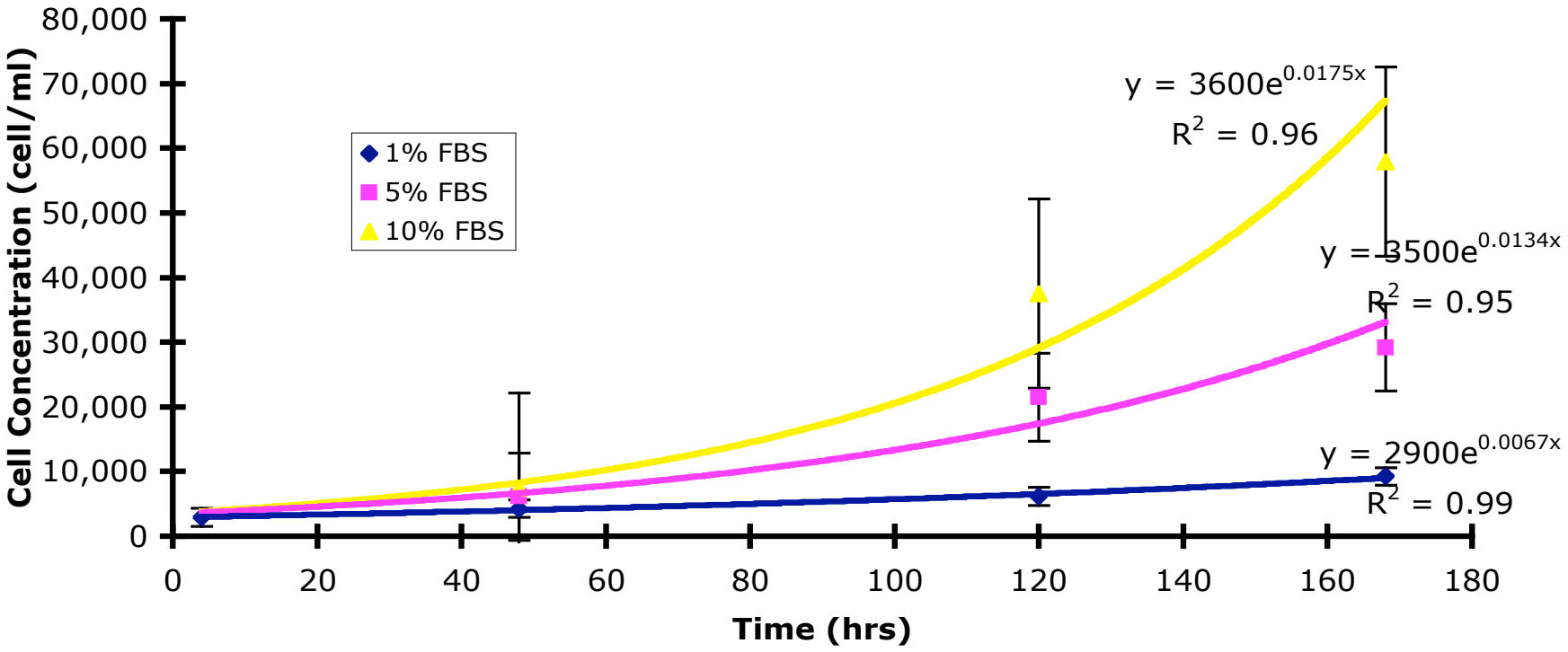
- MTT dye is reduced by metabolizing cells, producing a purple product. Absorbance increases when more MTT is reduced.
  - Since only viable cells have active metabolisms, the increase in absorbance indicates a greater number of viable cells.
- Cell concentration can be approximated from the linear relationship.

Percentage of Cells in S-phase Increases with Increasing Serum

FBS Conc.	**1%**	**5%**	**10%**
% of dividing cells	24% +/-6%	40% +/2%	50% +/-5%

- Nuclei of cells in S-phase were stained red.
- Percentage of proliferating cells increased with higher concentrations of FBS.

Increasing Serum Increases Rate of Cell Proliferation



FBS concentration significantly affects HDF concentration

- After 7 days, cells in 1%, 5%, and 10% FBS had significantly different concentrations (ANOVA, $p<0.001$).
- Doubling time decreases as FBS concentration increases.
  - 1%: 100 hrs
  - 5%: 50 hrs
  - 10%: 40 hrs
- Cell concentration increases more quickly in higher FBS concentrations since there is a greater percentage of proliferating cells.

Conclusions

- There is a linear relationship between HDF cell concentration and absorbance.
  - Unknown viable cell concentration can be estimated by this relationship.
- Increasing FBS concentration increases percentage of cells in S-phase, leading to lower doubling times, higher growth rates, and greater numbers of cells.
  - Long doubling times can restrict experimentation. Serum concentrations must yield cells quickly enough for projects to continue.