Human Dermal Fibroblast (HDF) Viability and Proliferation

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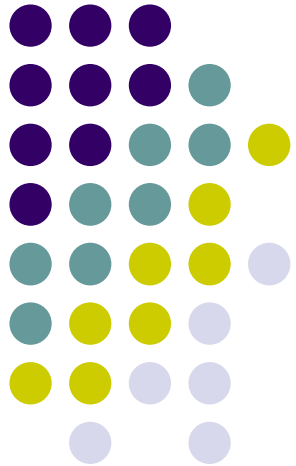
04/08/09



Objectives

- Observe and determine a linear relationship between absorbance and cell concentration using a MTT Assay.
- Determine the effect media serum concentrations has on cell proliferation using:
  - Anti-PCNA staining
  - Cell Proliferation Assay

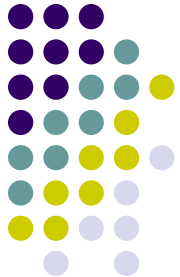
MTT Assay Methods

- The MTT assay is a metabolic assay that measures cells that are metabolizing.
- Cells were seeded in 7 wells at specific concentrations and incubated over 2 nights in DMEM with 10% FBS.
- Cell count was determined using the coulter counter for each well.
- Standard MTT dying procedures were followed using MTT dye, and solubilization/stop solution.
- Absorbance was determined using a Genesys 10UV Spectrophotometer.

Anti-PCNA Methods

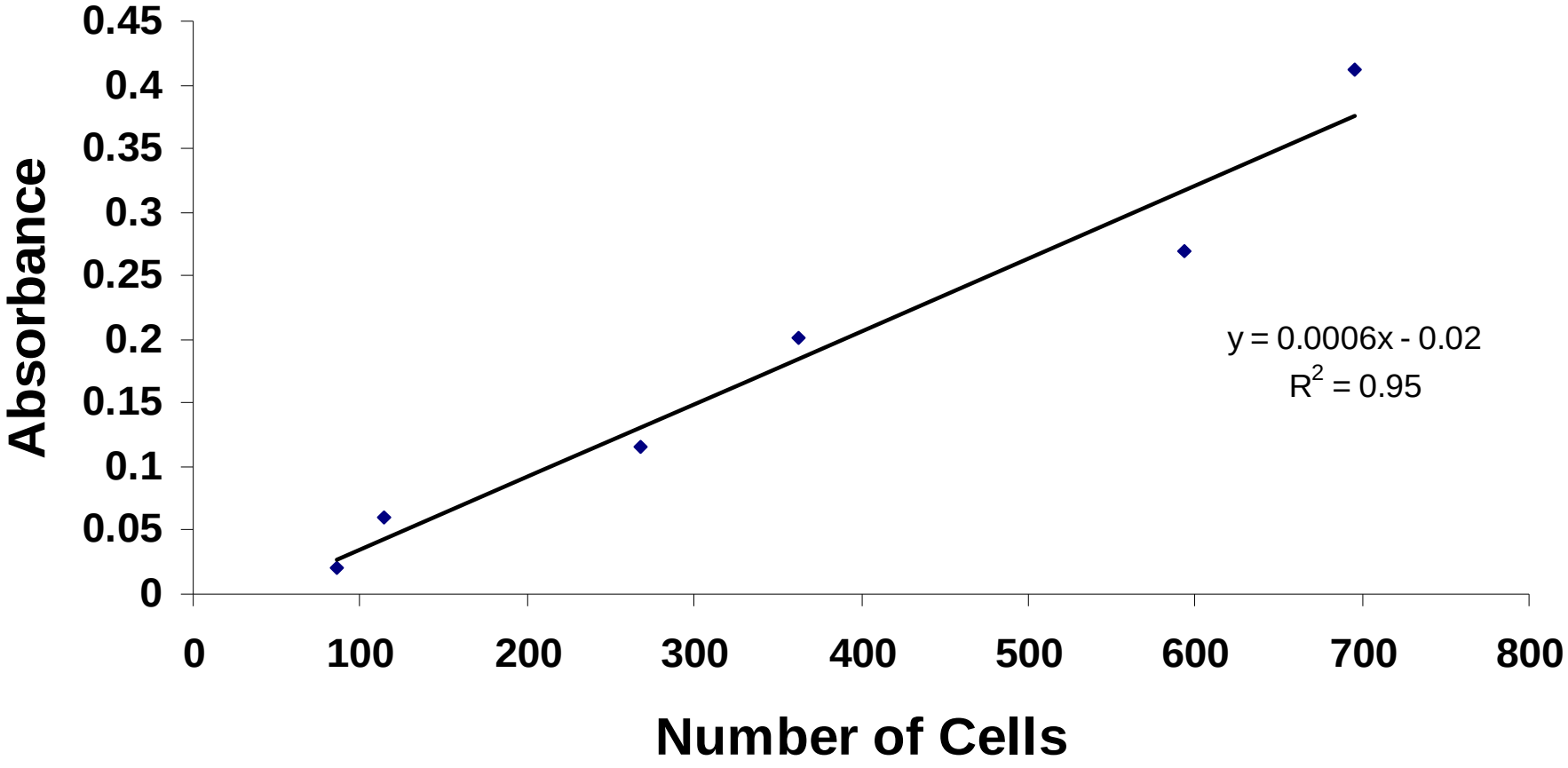
- Anti-PCNA staining is a antibody-specific procedure in which cells in S-phase (dividing) and not in S-phase (non-dividing) can be differentiated.
- Cells were seeded in 5 wells at a uniform concentration and were incubated over three days in DMEM media with 1%, 5%, and10% FBS (Fetal Bovine Serum), as well as positive and negative control wells.
- Standard staining procedures were followed, using anti-PCNA primary antibody, anti-mouse IgG secondary antibody, hematoxylin, and AEC solution to stain the nuclei of cells in S-phase.
- Stained cells were observed with a light microscope.

Cell Proliferation Assay Methods



- The cell proliferation assay is a way to determine the serum condition that allows for optimal cell growth and replication by looking at the cell number as a function of serum concentration and time.
- Cells were seeded into a total of 33 wells at a uniform concentration and allowed to incubate over 7 days in DMEM media with 1%, 5%, and10% FBS.
- Cell counts were taken using a coulter counter from each well condition on days 0,2,5,7.

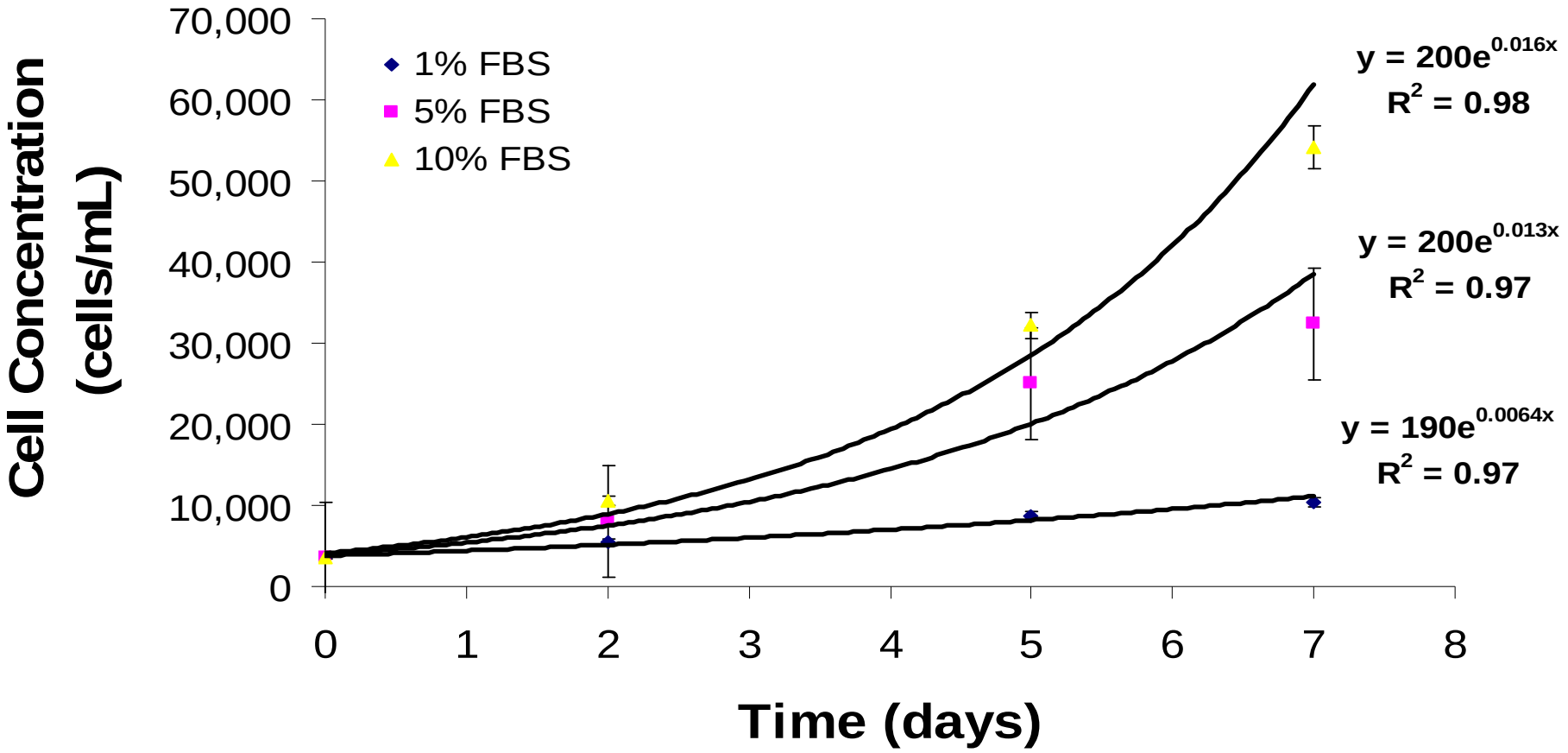
Cell Concentration and Absorption are Linearly Related



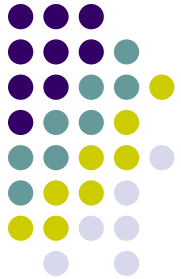
Higher FBS Concentrations Give More Cells in S-phase

Condition (%FBS)	% Red Nuclei	Observations
1%	0%	All cells were stained blue
5%	10%	More cell nuclei stained red
10%	30%	FBS concentration gave the most red nuclei per area

Cells Display Exponential Growth

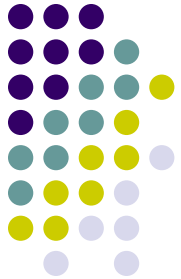


Cells Display Exponential Growth



- The graph shows that the greater the concentration of FBS, the greater the rate of growth of the HDF cells.
- The doubling times supports the finding that the cells in higher FBS concentrations doubled in less time. The doubling times are as follow: 108 hours for 1% FBS, 51 hours for 5% FBS, and 43 hours for 10% FBS.

Data Comparison: PCNA and Cell Proliferation Assay



- Assays complement one another
  - Both find that the rate and amount of cells growing and dividing depends on the FBS concentration.
  - The higher the FBS concentration, the faster cells grow and divide.

Summary

- Cell concentration and absorption are linearly related.
- The optimum FBS concentration that allows for greatest cell growth and division is 10% FBS.
  - This finding is supported by both assays.
    - PCNA found that the greatest percentage of cells in S-phase was when the cells were in 10% FBS.
    - Cell Proliferation assay showed that the cells were able to double the fastest when in the 10% FBS.