Human Dermal Fibroblasts: Viability and Proliferation Studies

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Goals of Viability and Proliferation Studies

- To use a colorimetric viability assay to determine the concentration of viable human dermal fibroblast (HDF) cells in a sample by creating a standard curve using known concentrations of cells.

- To determine the effect of media containing 1,5 or 10% fetal bovine serum (FBS) on the proliferation of HDF cells.

  - **Using coupled antibody assay to determine fraction of cells in S-phase**

  - **Using proliferation assay to determine growth rates.**

Creating a Standard Curve for Measuring Viable Cell Concentrations

- Two tissue culture (TC) treated 24-well plates were seeded with 0.5mL of a range of HDF concentrations and incubated @37ºC, 5% CO_2 for 2 days.
  - **0.00, 4,200, 8,300, 17,000, 25,000, 34,000, or 50,000 cells/mL**
- One well of each concentration was trypsinized, and concentration of cells was determined using a Coulter Counter.
- Remaining wells were treated with MTT die followed by the Solubilization/Stop solution.
- Contents of MTT treated wells were assayed for absorbance at 570nm, using the blank containing no cells as a zero.

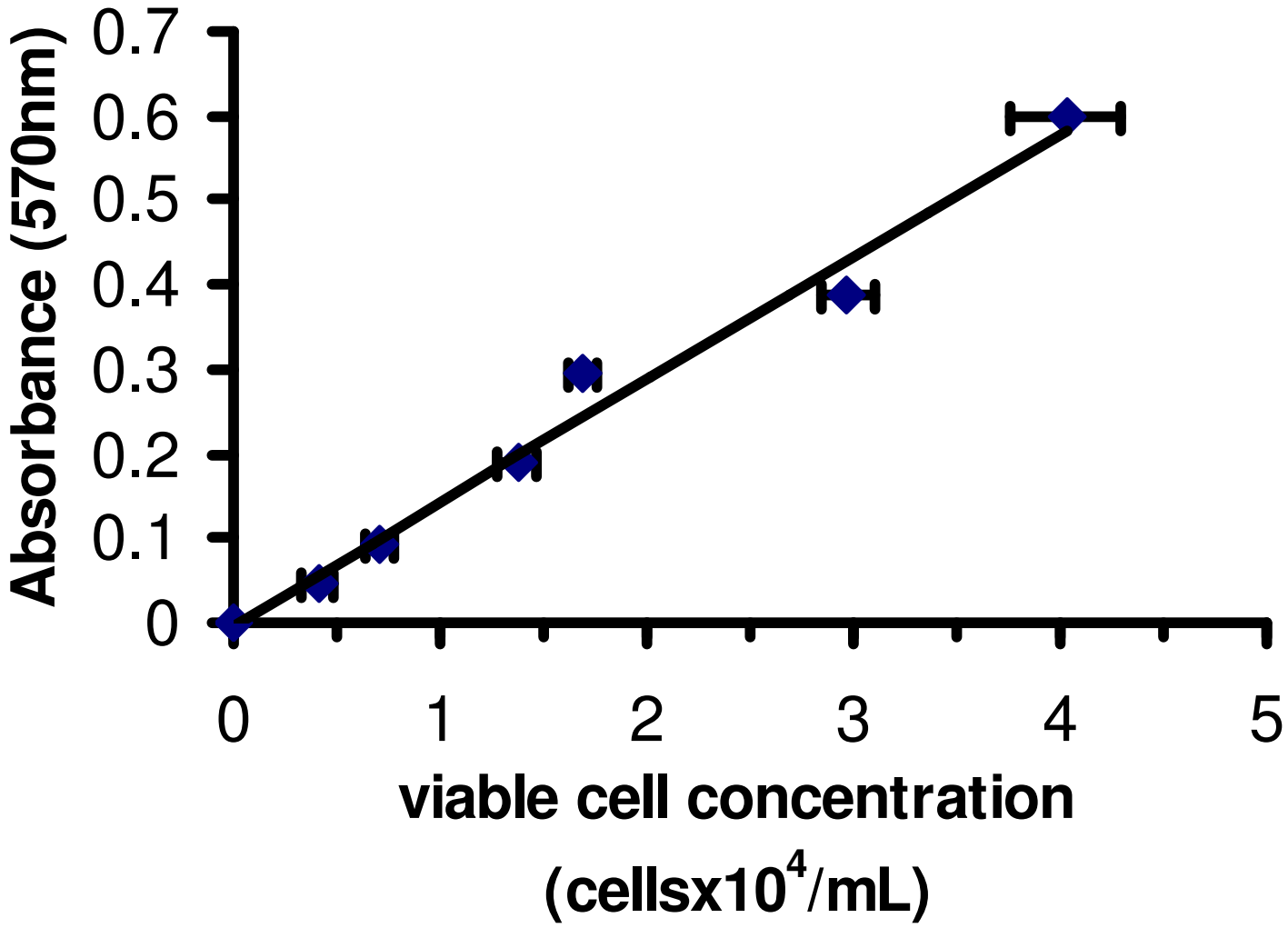
Determining Impact of Media Conditions on Fraction in S-phase

- Confluent cultures of HDF cells were harvested and diluted to 20,000 cells/mL in DMEM containing 1,5, or 10% FBS.
- A TC-treated 24-well plate was seeded with 1.0mL of 20,000 HDF cells/mL in DMEM containing varying FBS concentrations and incubated @37℃, 5% CO_2 for two days.
  - **1,5, or 10% FBS**
- Cells were affixed to wells with formalin and lysed with a methanol and hydrogen peroxide solution before being exposed to the primary antibody, anti-Proliferating Cell Nuclear Antigen (PCNA), and the secondary antibody, anti-Mouse IgG-Horse Radish Peroxidase (HRP).
- Antibody treated cells were then treated with AEC and hematoxylin, and visualized using a light microscope. Cells were classified as having either a red or blue nucleus, and controls were assayed for lack of red color.

Examining Effect of Media Conditions on HDF Growth Rates

- Two TC-treated 24-well plates were seeded with 1.0mL 5,000 HDF cells/mL with varing FBS concentration and incubated @37℃, 5% CO_2 for seven days.
  - **1,5, or 10% FBS.**
  - **6 wells were seeded for the t = 4 hour time point.**
  - **3 wells were seeded for the t = 2,5,7 day time points.**
- At appropriate time points, cells were trypsinized and counted in a Coulter Counter.

Colorimetric Response is Linear with Viable Cell Concentration

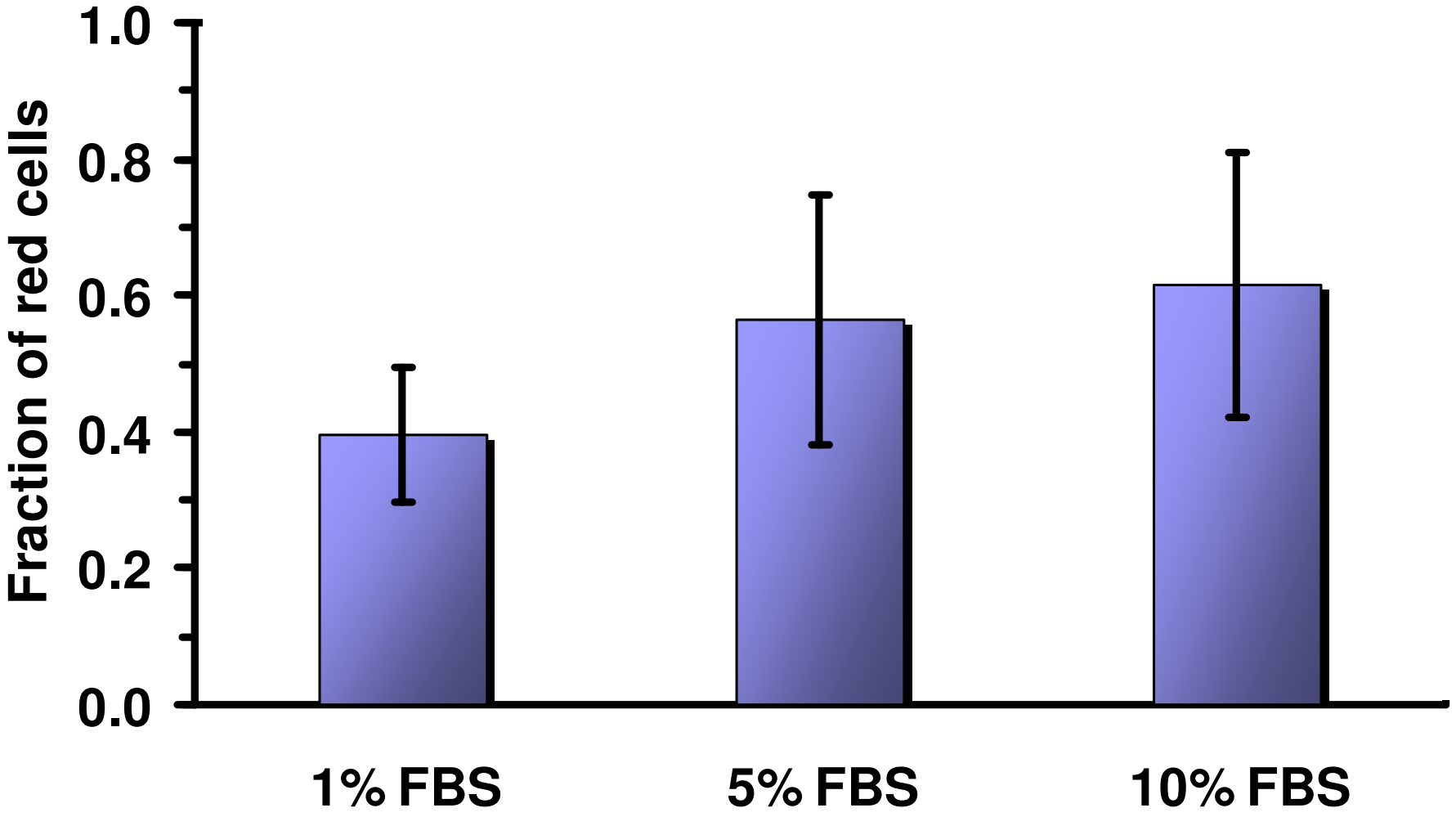


The Standard Curve for Absorbance vs. Cell Concentration is Linear

- Error bars represent the standard deviation obtained from measuring cell densities three times with the Coulter Counter.

- When the Absorbance @ 570nm is plotted against cell concentration and fit to a linear model, the line of best fit is $A_{570nm} = 1.5*10^{-5}(x)$, where x is the concentration of cells. The R^2 value for this line is 0.98, indicating a good fit to the observed data.

Fraction of Cells in S-phase Increases with FBS



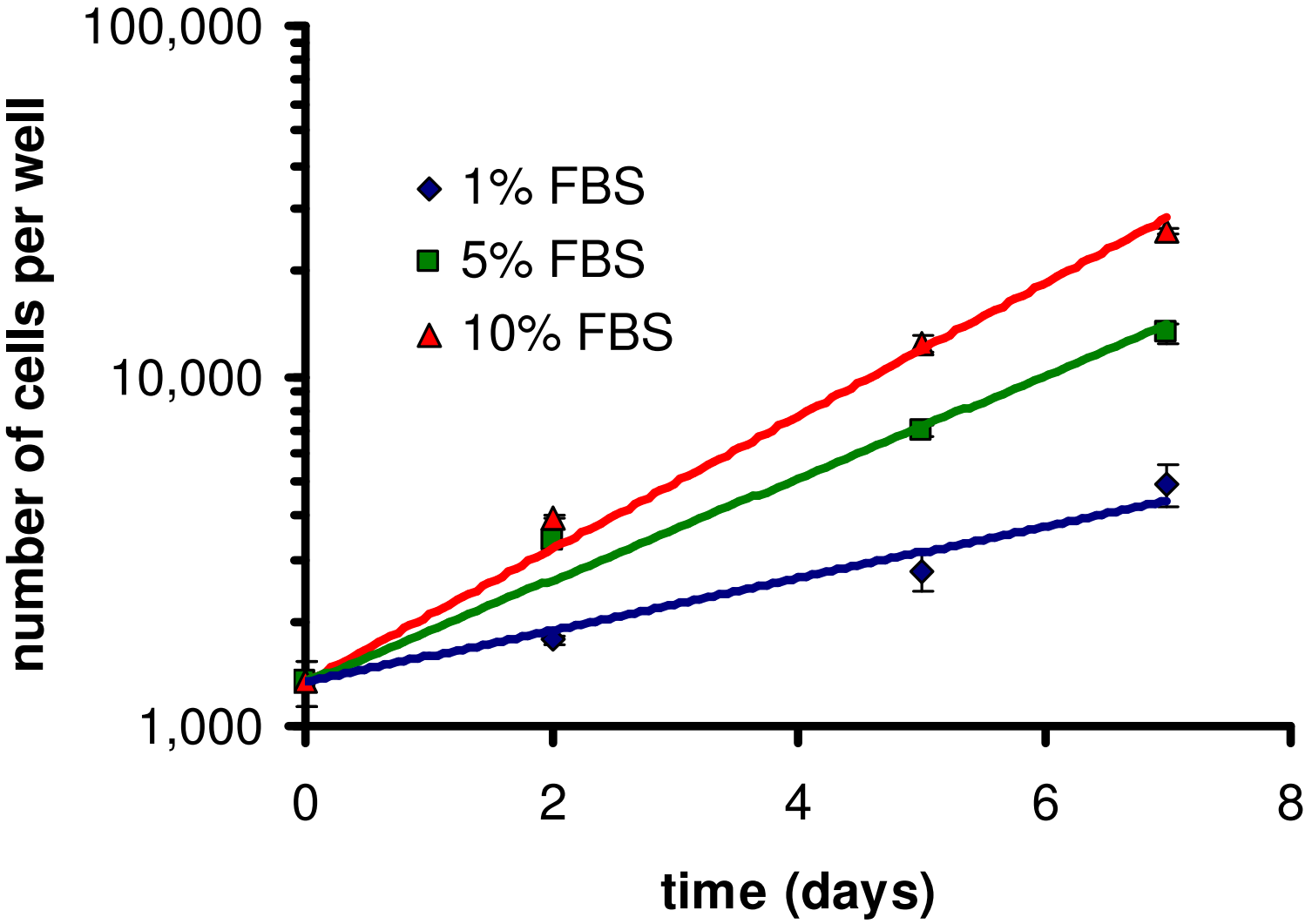
Data obtained from XXX

Fraction of Cells Committed to Mitosis Increases with FBS

- Error bars represent the range of data for number of samples = 2.
- Fraction of red cells increases with FBS concentration, suggesting that a higher fraction of cells are in S-phase, and actively going through the cell cycle at higher FBS concentrations.
- There appears to be a saturating effect as the difference between fraction of cells in S-phase between 1% and 5% FBS is greater than 5% and 10% FBS.

	Fraction of Cells in S-phase		
	1% FBS	5% FBS	10% FBS
mean	0.40	0.57	0.62
uncertainty	0.10	0.18	0.19

FBS Stimulates HDF Proliferation



FBS Increases Growth Rate of HDF

- The values and errors on the previous figure represent the average and standard deviations of three data points. The plotted curves represent the exponential of best fit and use the growth rates below.

- The growth rate of HDFs in 1% FBS media is significantly* lower than 5% or 10%, and 5% FBS is significantly* lower than 10%

FBS (v/v)	average rate (days^-1)	standard deviation (days^-1)	R^2 of fit
1%	0.44	< 0.01	0.97
5%	0.34	< 0.01	0.98
10%	0.17	0.01	0.98

- There appears to be a saturating effect in that the growth rate had a smaller increase from 5% to 10% than from 1% to 5%.

*Significance is defined as $p<0.05$ generated from a single factor ANOVA

Fraction of Cells in S-phase Correlates to Growth Rate

- The anti-PCNA assay shows that at higher FBS concentrations, there is a higher fraction of cells in S-phase, moving through the cell cycle. This correlates to the increased growth rate of cells in higher FBS concentrations, implying that a higher percentage of cells moving through the cell cycle results in a higher growth rate.

- Both the anti-PCNA and cell proliferation assay showed evidence of a saturating phenomenon where fraction of cells moving through the cell cycle and growth rates appeared to plateau around 10% FBS. This implies that 10% FBS may be sufficient to fully stimulate cell proliferation in HDF cells grown in DMEM.

Conclusions

- There is a linear relationship between the concentration of viable cells and the colorimetric response produced by the MTT assay, allowing for determination of concentration of viable cells.
- Increasing FBS concentration causes a higher fraction of cells to be in S-phase, actively participating in the cell cycle. There does appear to be a saturation of fraction of cells in S-phase around 60%.
- FBS stimulates HDF proliferation, increasing the cellular growth rate. There does appear to be a saturation of growth rate around 0.44, or, a doubling time of 1.5 days.