

Growth and Replication of HDF cells

6/2/08

Objectives

- Visualize a linear relationship between absorbance and concentration
- Observe a connection between cell cycle/proliferation and media conditions
- Assess the effects of serum percentages on the growth and replication of HDF cells

MTT Viability Test Methods

- Seed 2 plates with 7 different cell concentrations from 0 cells/mL to 50,000 cells/mL
- Incubate for 2 nights
- For CC plate: Trypsinize wells, obtain concentrations using coulter counter
- For MTT Dye plate: Add dye solution, wrap plate in foil, incubate 2 hrs, add stop solution, record absorbance for each well using spectrophotometer
 - Yellow dye solution is reduced to purple by live cells
 - Spectrophotometer will assess the absorbance of the purple solution which is equivalent to the number of live cells

Anti-PCNA Methods

- Seed all wells with cell concentration of 20,000 cells/mL
 - Conditions A, B, C get 1, 5, 10% FBS respectively
 - Controls get 10% FBS
- Incubate for 2 days
- Rinse wells, expose them to primary then secondary antigen
 - Controls do not get both antigens
- Add AEC then hematoxylin
- View with light microscope
 - Cells in S Phase will soon replicate and are stained red
 - Other cells are stained blue

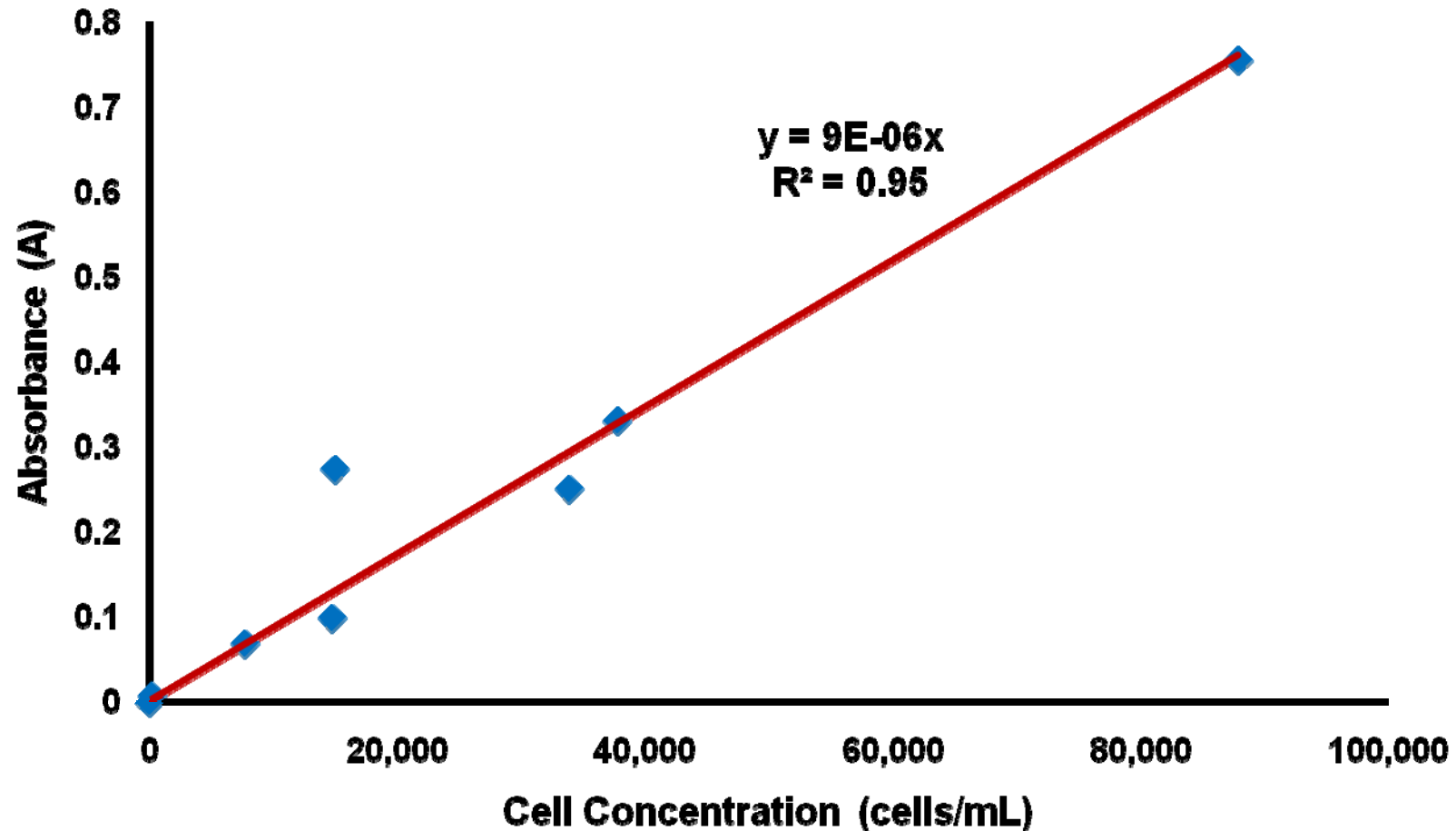
Cell Proliferation Methods

- Seed wells with 5,000 cells/mL suspension
 - Day 0 wells have 1% FBS
 - Rest of wells have 1, 5, or 10% FBS as the test condition
- Obtain cell concentration for each condition at 4 hrs and 1, 3, and 7 days after seeding
 - Rinse and trypsinize designated wells
 - Record concentration using coulter counter

Absorbance Decreases with Decreasing Cell Concentration

Well	Vol. of Cells (mL)	Vol. of Media (mL)	Concentration (cells/mL)	Absorbance (A)
1	0.5	0	87,960	0.756
2	0.2	0.3	37,800	0.331
3	0.167	0.333	33,920	0.251
4	0.125	0.375	15,040	0.274
5	0.071	0.429	14,800	0.099
6	0.038	0.462	7,760	0.069
7	0	0.5	240	0.007

Concentration and Absorbance are Linear



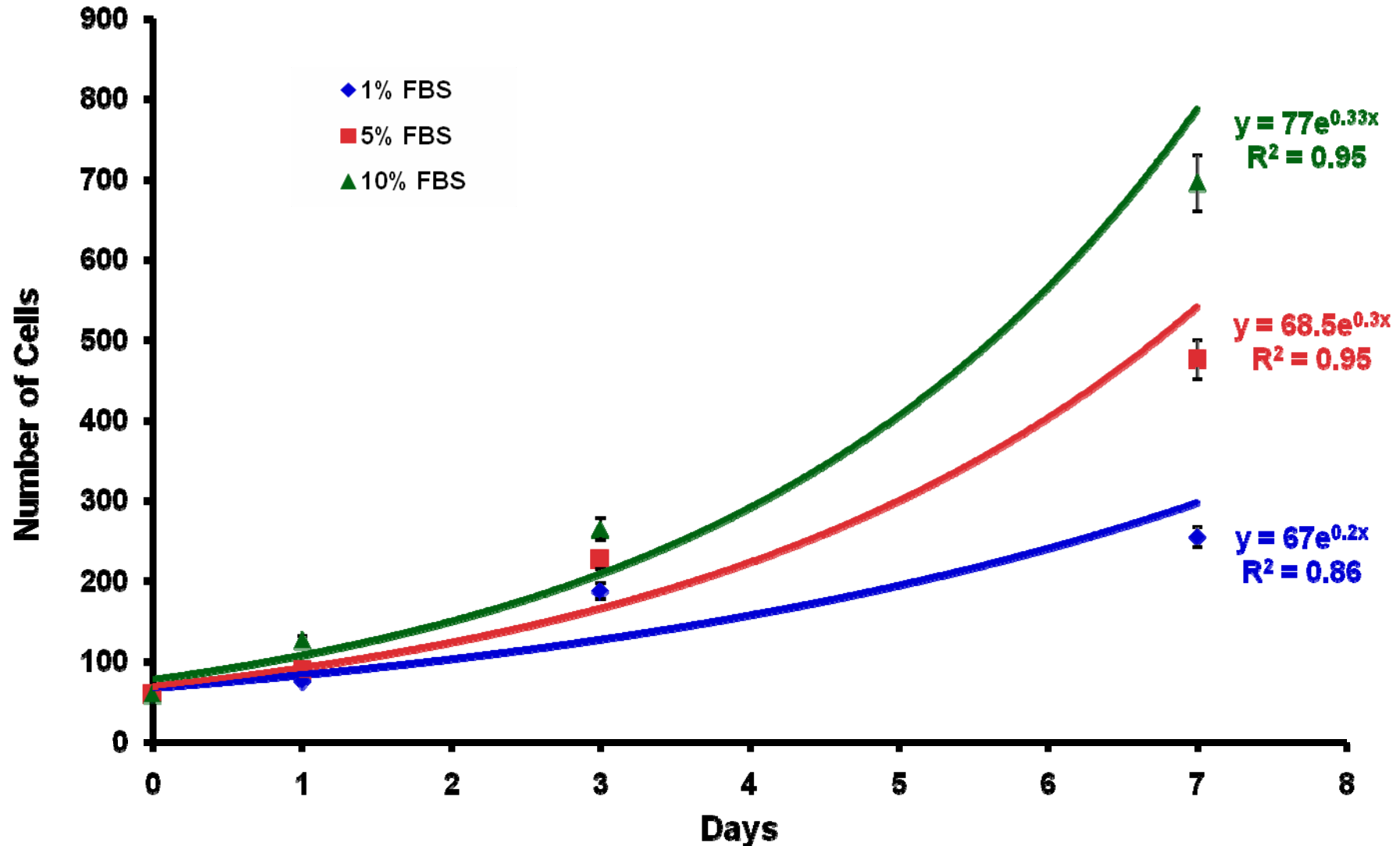
- Accurate linear relationship ($R^2 = 0.95$)
- Higher concentration yields higher absorbance and more live cells

Higher FBS Percentage Yields More Cells in S Phase

	Variable	Nuclei Color
Condition A	1% FBS	50% red
Condition B	5% FBS	60% red
Condition C	10% FBS	80% red
Control 1,2, & 3	---	100% blue

- Controls show that cells in S Phase cannot be tagged without being subjected to both the primary and secondary antigens
- As % FBS increases from 1 to 10, the number of cells in S Phase increases

Exponential Cell Proliferation



*Data from XXX

10% FBS Yields Highest Proliferation Rate

- Cells in all 3 test conditions show exponential growth
 - Treadlines fit well - R^2 values 0.95, 0.95, 0.86
- Starting at the same cell concentration, by day 7 the three conditions show large differences in concentration
 - Cells with 10% FBS show the greatest growth rate
 - The 5% FBS yields a moderate growth of cells
 - Growth rate with 1% FBS is much lower

Anti-PCNA and Cell Proliferation

- Anti-PCNA Assay: Media with 10% FBS results in the highest percentage of cells in S Phase
 - Cells in S Phase initiate replication
- Cell Proliferation Assay: Cells grown in media with 10% FBS grow at the fastest rate
- Growing cells in media with 10% FBS results in a higher percentage of replication initiation and therefore a faster proliferation rate

Conclusions

- Cell concentration has a linear relationship with absorbance
- Cells grown in 10% FBS have the most cells in S Phase after 2 days
 - Followed by cells grown in 5% FBS
 - Cells grown in 1% FBS have the least
- 10% FBS results in a larger exponential growth than 1% and 5%