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# Serum Effects & the Absorbance - Concentration Relationship for HDF Cells

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BIOE 342

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# Experimental Objectives

- Quantitatively assess the effects of FBS on HDF proliferation
  - Cell Proliferation Assay
- Determine qualitative effects on FBS on proliferation and the number of cells in the cell cycle
  - Anti-PCNA Staining
- Develop a relationship between absorbance and cell concentration
  - MTT Viability Assay

# Cell Concentration & Absorbance Measurements

- HDF passage 8 cells seeded in duplicate in DMEM with 10% FBS at tabulated concentrations
- Cells incubated for 2 days
- Concentrations of one set measured with Coulter Counter
- Other set treated with MTT dye and absorbance measured with spectrophotometer

Concentration (cells/ml)
50,000
33,500
25,000
16,700
8,300
4,170
0 (control)

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# Proliferation Measurements

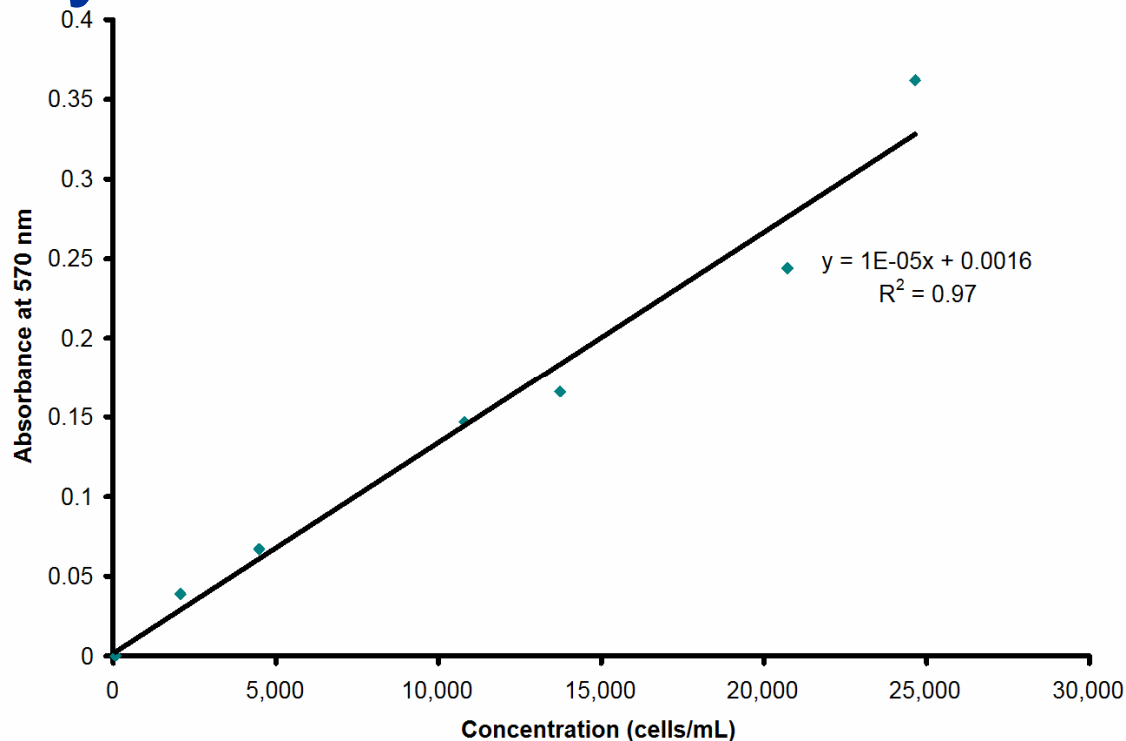
## Cell Proliferation Assay

- Seeded HDF p. 9 cells in DMEM with 1% FBS
- After 4 hrs incubation, Day 0 wells measured (n=5)
- Remaining cells grown in DMEM (1, 5, or 10% FBS; n=3)
- Day 2, 7 & 5 attached cells counted with Coulter Counter

## Anti-PCNA Staining

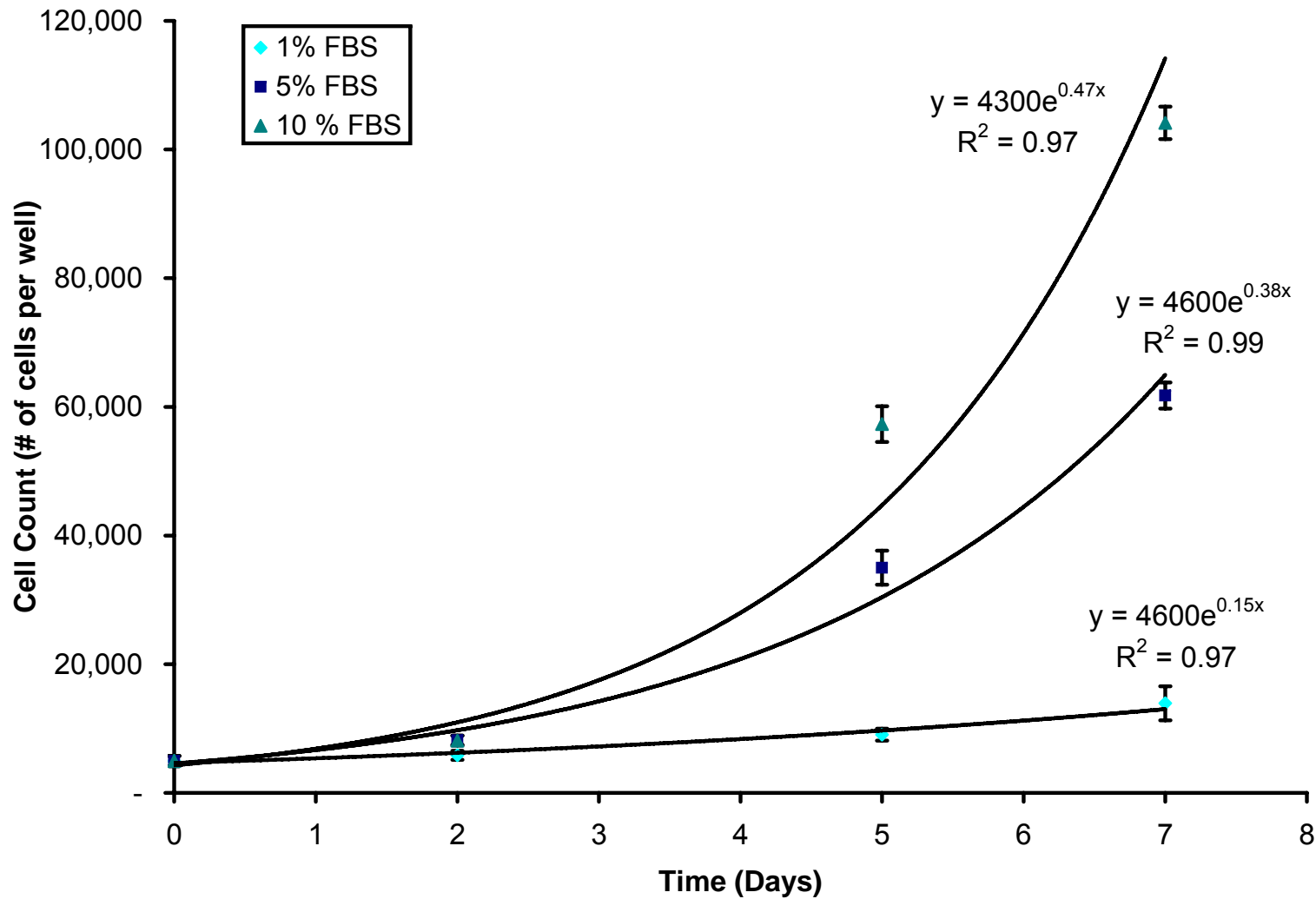
- Seeded HDF p. 9 cells in DMEM (1, 5, or 10% FBS)
- After 2 days incubation, Anti-PCNA added
- 3 test wells received 1°, 2° antibodies
- 3 control wells received 1°, 2°, or no antibody
- Noted nuclei color and confluency with light microscopy

# Absorbance and Cell Concentration Linearly Related



- Saturation does not occur from 0 to 25,000 cells/ml
  - Saturation would reduce linearity at high values

# Cell Growth Occurs Exponentially



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# Exponential Model Reveals Doubling Time & FBS Levels Inversely Related

- Proliferation may be modeled exponentially for all serum levels ( $R^2 \geq 0.97$ )
- Cell counts at 2, 5 & 7 days in 10% FBS levels all statistically different (ANOVA, Tukey's,  $p < 0.05$ )
  - Statistical difference strengthens exponential model
- Cell counts at each FBS level statistically different after 7 days (ANOVA, Tukey's,  $p < 0.05$ )
  - Positive relationship between FBS level & cell count
- Doubling times calculated as 4.6, 1.8 & 1.5 days for 1, 5 & 10% FBS respectively

# FBS Increases Fraction of Red Nuclei

- At all conditions:
  - 95% cells elongated & spread, <5% rounded

	Condition	Nuclei Color (%)		Confluency (%)
		Red	Blue	
Tests	1% FBS	33	67	40
	5% FBS	45	55	60
	10% FBS	60	40	70
Controls	1° Antibody	0	100	70
	2° Antibody	0	100	70
	No Antibody	0	100	70



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# Number of Cells in Cell Cycle Positively Related to FBS Level

- Red corresponds to PCNA presence
  - PCNA is abundant in nucleus during S Phase
  - More cells prepare for mitosis at higher FBS levels
  - Number of cells in  $G_0$  decreases as FBS increases
- Confluency increased with FBS levels
  - Proliferation increases as FBS concentration increases
- No red nuclei appeared in controls
  - Intrinsic peroxidase activity is insignificant
  - 2° antibody nonspecific binding is insignificant

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# FBS Increases Proliferation

## Quantitatively from Cell Proliferation Assay

- Statistically significant increase in cell count from 14,000 to 100,000 cells/ml at 7 days as FBS increased from 1 to 10%

## Qualitatively from Anti-PCNA Staining

- Increase in confluency from 40 to 70% suggests increased proliferation
- Fraction of cells in S phase increased from 33 to 60% as FBS increased from 1 to 10%
- Increased number of cells in the cell cycle predicts increased proliferation found in quantitative assay

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# Serum Effects & Absorbance-Concentration Relationship

- Absorbance and cell concentration found to have a linear relationship ( $R^2=0.97$ )
- Proliferation may be modeled exponentially ( $R^2\geq 0.97$ )
- At 7 days, a positive relationship between FBS level & cell count existed (ANOVA, Tukey's,  $p < 0.05$ )
- Doubling times decreased (4.6, 1.8 & 1.5 days) for 1, 5 & 10% FBS respectively
- Increase in percentage of red nuclei as FBS level is increased reflects an increase in number of cells in S-phase