

Tissue Culture: Viability and Proliferation Studies

Purpose

- **MTT Viability Assay:** To verify that there is a linear relationship between absorbance and cell concentration
- **Anti-PCNA Staining:** To determine the connection between media conditions and cell division
- **Quantitative Growth Proliferation Assay:** To assess the effects of serum on growth and replication of cells

Methods for the MTT Viability Assay

- **Stock HDF (Human Dermal Fibroblast) solution diluted with DMEM to six different concentrations**
- **Incubated for 48 hours on 24 well plate**
- **Cell concentration confirmed via Coulter counter**
- **Absorbance reading measured using MTT dye solution and spectrophotometer (570 nm)**
- **Measured and recorded absorbance as a function of concentration**

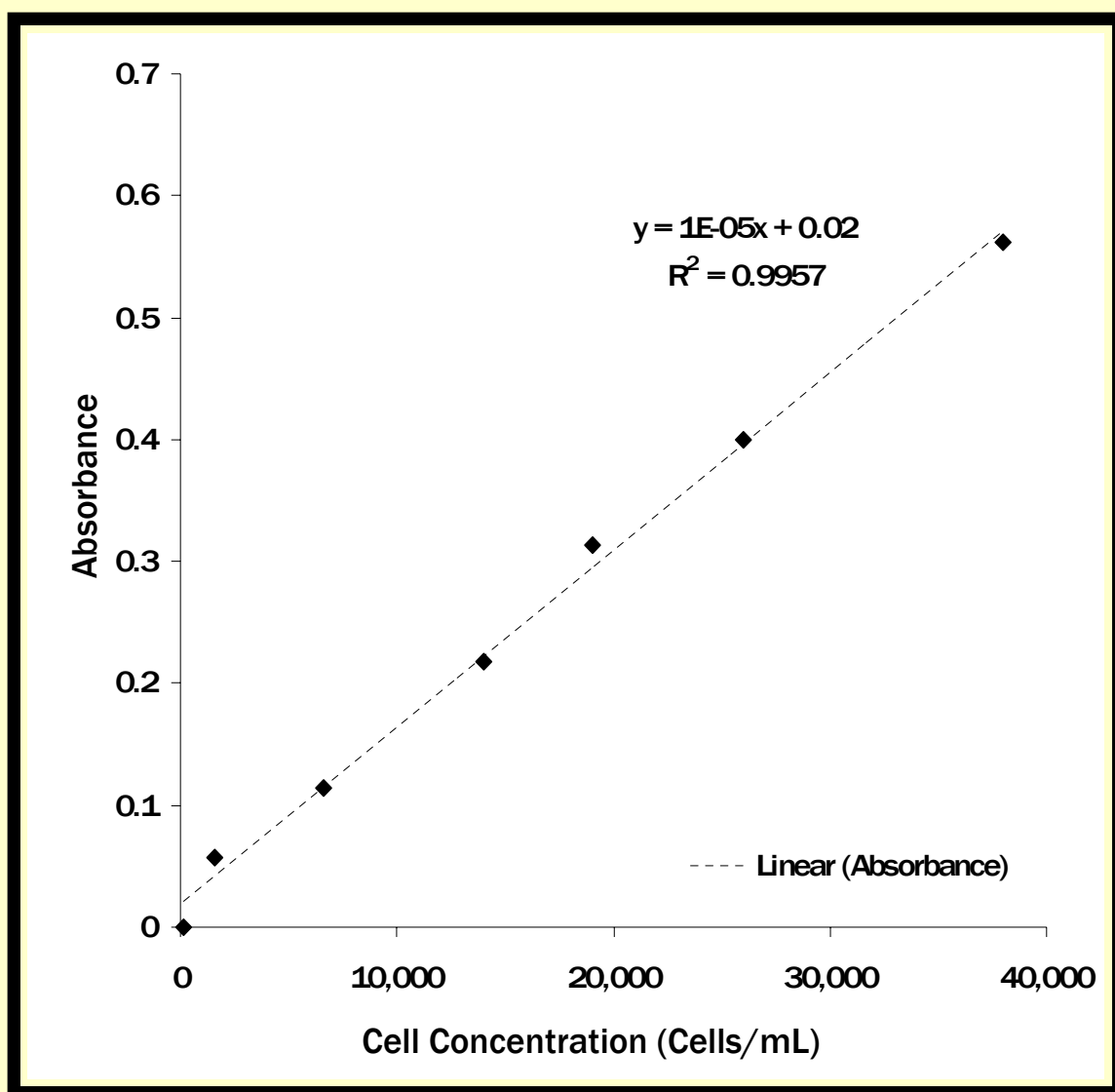
Methods for Anti-PCNA Staining

- Incubated HDF cells for 48 hours under one of three conditions: DMEM with 1% antibiotic and 1, 5, or 10% serum
- Added Anti-PCNA primary antibody, then Anti-mouse IgG Horseradish Peroxidase (HRP) secondary antibody to cells
- Added Aminoethyl carbazole (AEC) and hematoxylin for staining
 - AEC binds to horshradish peroxidase to stain dividing cell nuclei red
 - Hematoxylin stains all cell nuclei a blue color
- Using light microscope, estimated percentage of cells that are preparing to divide for each condition (% of red nuclei)

Methods for Quantitative Cell Proliferation Assay

- **Week-long study with 3 observation points**
- **Seeded HDF cells on 24 well plate in DMEM with 1% serum and incubated for 4 hours**
- **Switched media to 1 of 3 conditions: DMEM with 1, 5, or 10% serum**
- **At each observation point (2, 5, and 7 days), cells trypsinized and cell count recorded using Coulter counter, then converted to concentration**

MTT Assay: Linear Relationship for Absorbance vs. Concentration



Concentration (Cells/mL)	Absorbance
38000	0.562
26000	0.4
19000	0.313
14000	0.218
6600	0.114
1600	0.057
160	0

- The R^2 value of the linear fit is 0.9957
- This strongly suggests that absorbance and concentration are linearly related

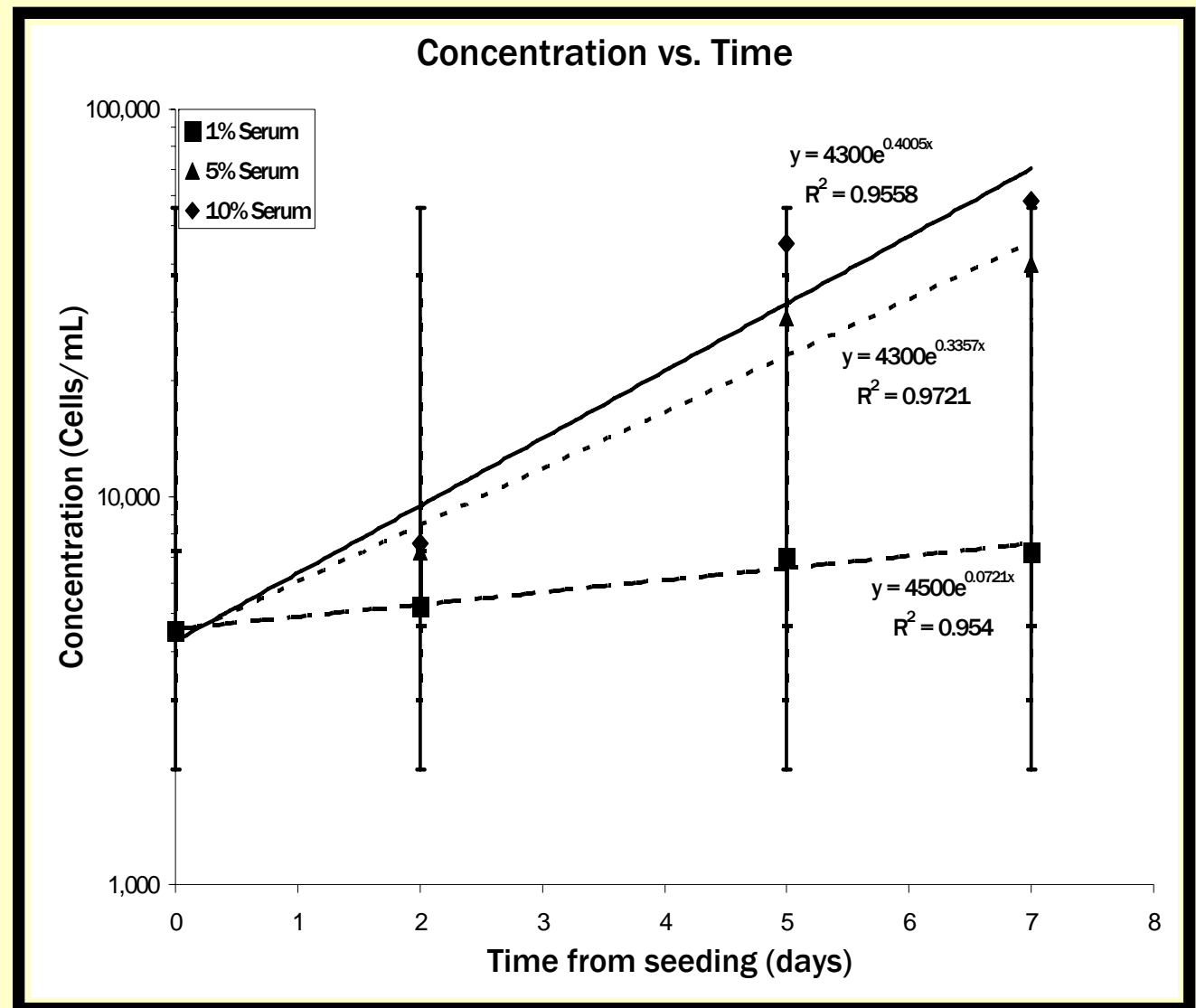
Anti-PCNA Staining: More Serum = More Cell Division

Serum Percentage	Percent of nuclei staining red
1%	40%
5%	70%
10%	80%

- **All results are based upon human observation, where red nuclei reflect cells preparing to divide**
- **Elevated levels of serum result in more availability of nutrients required for cell division**
- **Thus higher levels of serum promote a higher percentage of cells undergoing cell division, explaining the increasing percent of nuclei staining red**

Cell Proliferation Assay: Cell Growth is Exponential Under Ideal Conditions

- After initial cell seeding and before full confluency/media exhaustion, growth in each condition was exponential
- R^2 values close to 1 for each condition support this conclusion



Cell Proliferation Assay: Doubling Rate is Inversely Related to Serum Percentage in Media

Doubling Time from Graph

Condition	Trendline Equation	k value	Doubling Time (hours)
1%	$4600e^{0.003x}$	0.003	230
5%	$4300e^{0.014x}$	0.014	50
10%	$4300e^{0.0167x}$	0.0167	42

Doubling Time from Exponential Growth Equation

Day	Doubling time (hours)		
	1% Serum	5% Serum	10% Serum
2	230	71	63
5	188	45	36
7	248	53	46
Average	222	56	48

- Doubling rate was calculated in two ways: via the exponential trendline equation and via manipulation of the equation

- $N(t) = N_0 * 2^{tf}$ where $N(t)$ is number of cells after t days, N_0 is initial cell count, and f is $1/\text{doubling time}$

- Both of these methods provide us with similar answers, as the tables indicate

Anti-PCNA Staining vs. Cell Proliferation Assay

- **Each case displayed an observable trend**
- **As percentage of serum increases in DMEM, cell division levels increase**
 - **Observable directly in Anti-PCNA staining**
 - **Implied via doubling times in Proliferation Assay**
- **Anti-PCNA staining more accurate, considering that Coulter counter used in Proliferation Assay may not have only counted cells (detritus, etc.)**

Experimental Summary

- **MTT Assay concluded that there is a linear relationship between cell concentration and absorbance at 570 nm for HDF cells**
- **Anti-PCNA Assay and Cell Proliferation Assay concluded that increasing levels of serum will increase the rate at which cell division occurs, and that**
- **Cell Proliferation Assay concluded that cellular growth is exponential under ideal conditions**