Human Dermal Fibroblast Cell Survival and Function *in Vitro*

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Objectives

- Find the relationship between proliferation and fetal bovine serum (FBS) concentration in media
  - Anti-PCNA Staining
  - Quantitative Cell Proliferation Assay
- Observe the impact of a toxic material on HDF cells
  - Live/Dead Fluorescence Assay
- Find the relationship between absorbance and cell concentration
  - MTT Test

Anti-PCNA Staining

- Seeded each well with 20,000 HDF cells
  - Experimental wells (varied serum): DMEM + 1, 5, or 10% FBS
  - Control Wells: DMEM + 10% FBS
- Conducted antibody staining assay to identify cells with Proliferating Cell Nuclear Antigen (PCNA):

- Observed cells under light microscope for color and confluency
  - All cells stain blue
  - Cells in S phase have nuclei stained red

Quantitative Cell Proliferation Assay Methods

- Seeded each well with 5,000 HDF cells
  - Experimental wells (varied serum): DMEM + 1, 5, or 10% FBS
- Replenished media on days 2 and 5 after seeding
- Measured cell concentrations using Coulter Counter at 4 hours and 2, 5, 7 days after seeding

Viability Assay Methods

Live / Dead Assay

- Seeded HDF cells and incubated for 2 days
- Created 3 test conditions
  - PBS (Control) + dye
  - EtOH + dye
  - PBS + EtOH (drops) + dye
- Observed color of cells under fluorescence microscope
  - Live cells stain green
  - Dead cells stain red

MTT Test

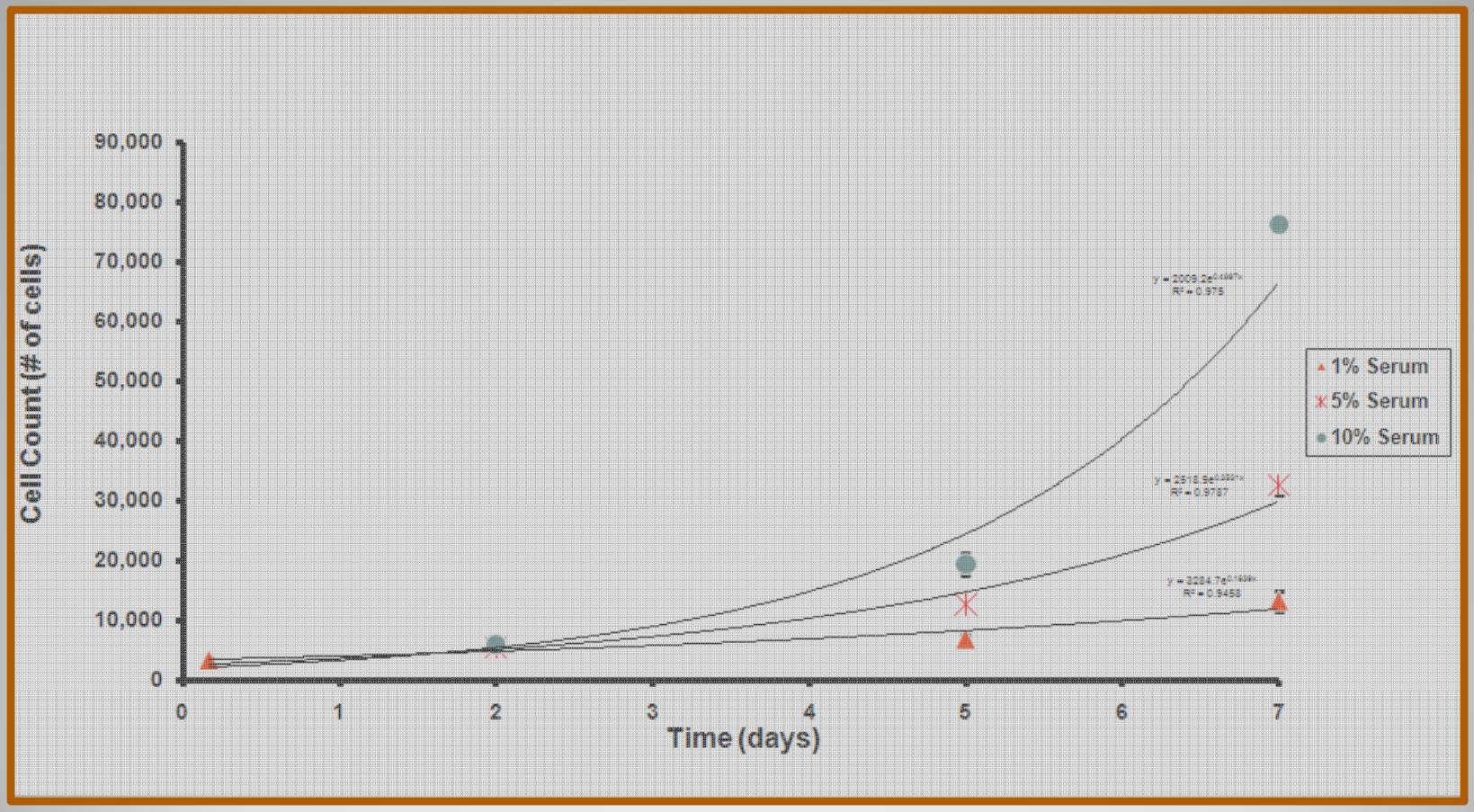
- Seeded wells on 2 separate plates with different numbers of HDF cells (2,000-25,000 cells) and incubated for 2 days
- Conduced metabolic assay on plate 1
  - MTT dye added to each well
  - Recorded absorbance of samples using a spectrophotometer
- Measured cell concentrations of plate 2 using Coulter Counter

PCNA Results: Serum Promotes S Phase

Test Condition	Confluency	Color of Nuclei
Controls	80-90%	0% red
1% FBS	65%	50% red
5% FBS	85%	75% red
10% FBS	90%	95% red

- As serum concentration increased, we observe:
  - Greater cell density
  - Greater fraction of cells in S phase

Cells Grow Exponentially with Serum

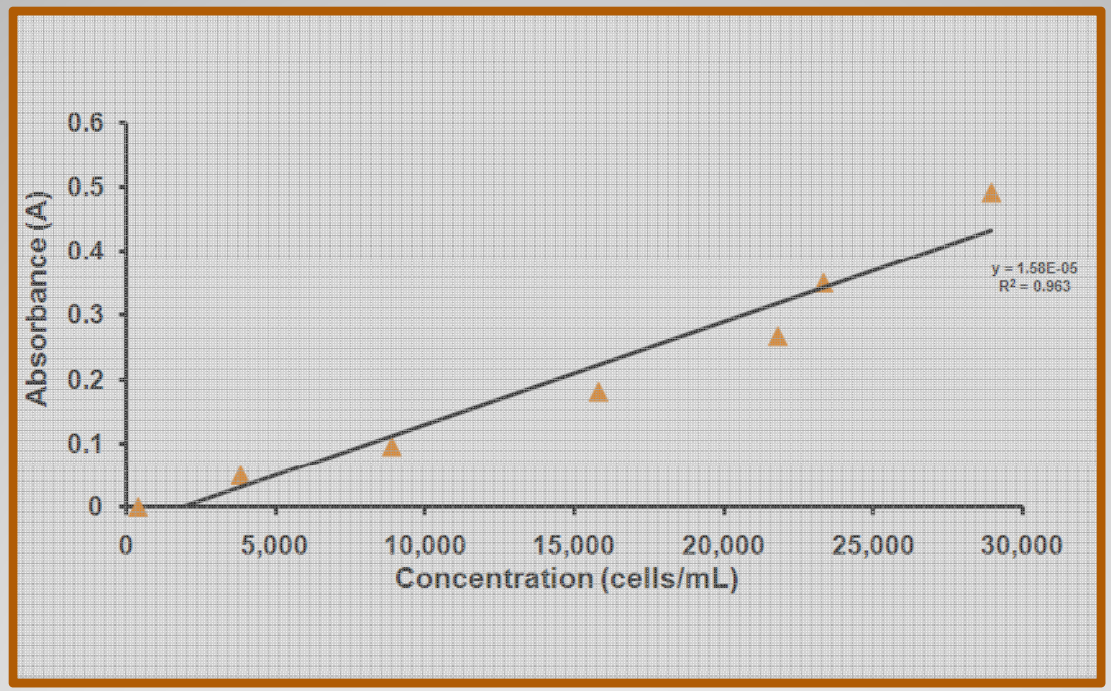


Proliferation Assay: Cell Doubling Times

Test Condition	Cell Doubling Time (Days)
1% FBS	2.57
5% FBS	0.78
10% FBS	0.61

- As serum concentration is increased, we observe:
  - Cell doubling time decreases
  - Growth rate increases

Absorbance and Concentration are Related Linearly



- The relationship between the absorbance and concentration is linear and statistically significant (ANOVA, $p<0.005$)

Live / Dead Assay Confirms Toxicity of Ethanol

Test Condition	Confluency	Viability
PBS	85%	All live cells
EtOH	70%	All dead cells
PBS + EtOH (drops)	85%	Cells dead in EtOH areas

- Ethanol is a cytotoxic substance
  - Wells with ethanol had large amounts of dead cells

Summary: Proliferation Assays

Cell Proliferation Assay:

- Greater concentrations of serum yield greater proliferation rates
  - Serum contains growth factors and essential amino acids that promote continuation of the cell cycle

Anti-PCNA Stain:

- Greater concentrations of serum yield greater fractions of cells in S phase
  - Greater concentrations of serum yield greater proliferation rates
  - High proliferation rates means more cells undergoing mitosis
  - The S phase prepares cells for mitosis
  - Populations with high proliferation rates have a greater fraction of cells in S phase

Summary: Viability Assays

Live/Dead Assay:

- Ethanol is cytotoxic
  - Assay enables observation of both live and dead cells
  - Easy to determine cytotoxicity of a substance
  - Large numbers of cells died when EtOH present

MTT Test:

- Absorbance and Viable Cell Concentration are Linearly Related
  - Concentration easily determined using linear interpolation