HDF Cell Viability and Proliferation in vitro

February 13, 2008

Experimental Objectives

- To analyze relationship between live, metabolically active cells and absorbance
- To assess toxicity of ethanol and PBS on HDF cells, to observe staining of live and dead cells
- To quantitatively assess effects of serum on HDF cell growth and replication

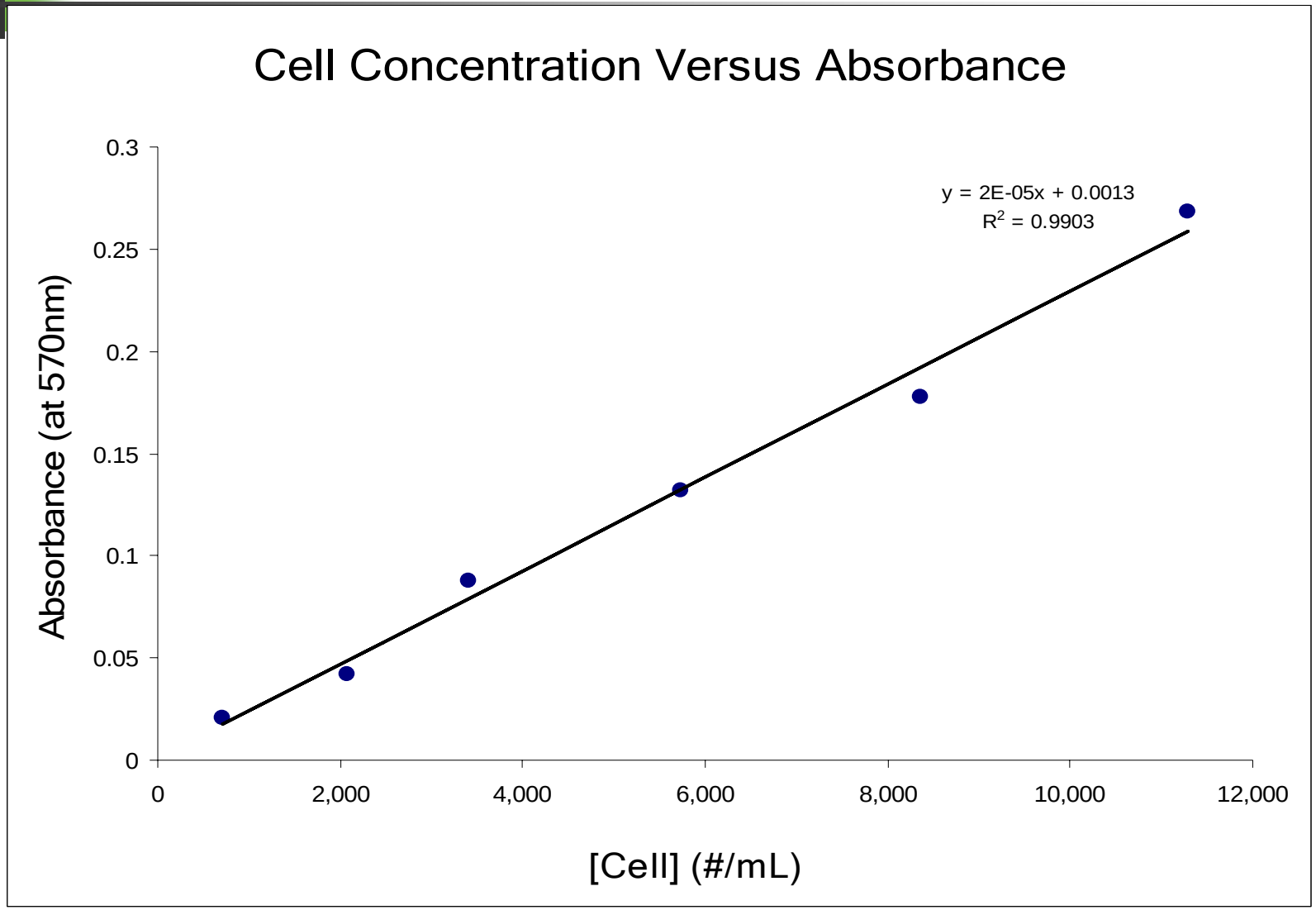
Viability Assays Experimental Procedure

- MTT Viability Assay
  - Test 6 samples of HDF cells at different dilutions
  - Obtain cell count with Coulter Counter
  - Incubate samples with MTT dye, then with Solubilization/Stop solution
  - Use spectrophotometer to read absorbance at 570 nm

Viability Assays Experimental Procedure

- Live/Dead Fluorescence Assay
  - Treat HDF cell samples with 3 conditions
    - 1 – PBS, dye
    - 2 – ethanol, dye
    - 3 – PBS + ethanol, dye
  - Use fluorescent microscope to observe red (dead) and green (live) stained cells

Linear Relationship between Cell Concentration and Absorbance



PBS is Harmless and Ethanol is Lethal to HDF Cells

Condition	Observations
1 – PBS dye	▪All cells green (live) and attached ▪Spread, pseudopodia visible
2 – ethanol dye	▪All cells stained red (dead) and attached ▪No pseudopodia
3 – PBS, ethanol dye	▪High density of red stained cells in center of well, surrounded by green stained cells ▪Dead cells attached ▪About 50% of live cells attached, 50% detached and spherical

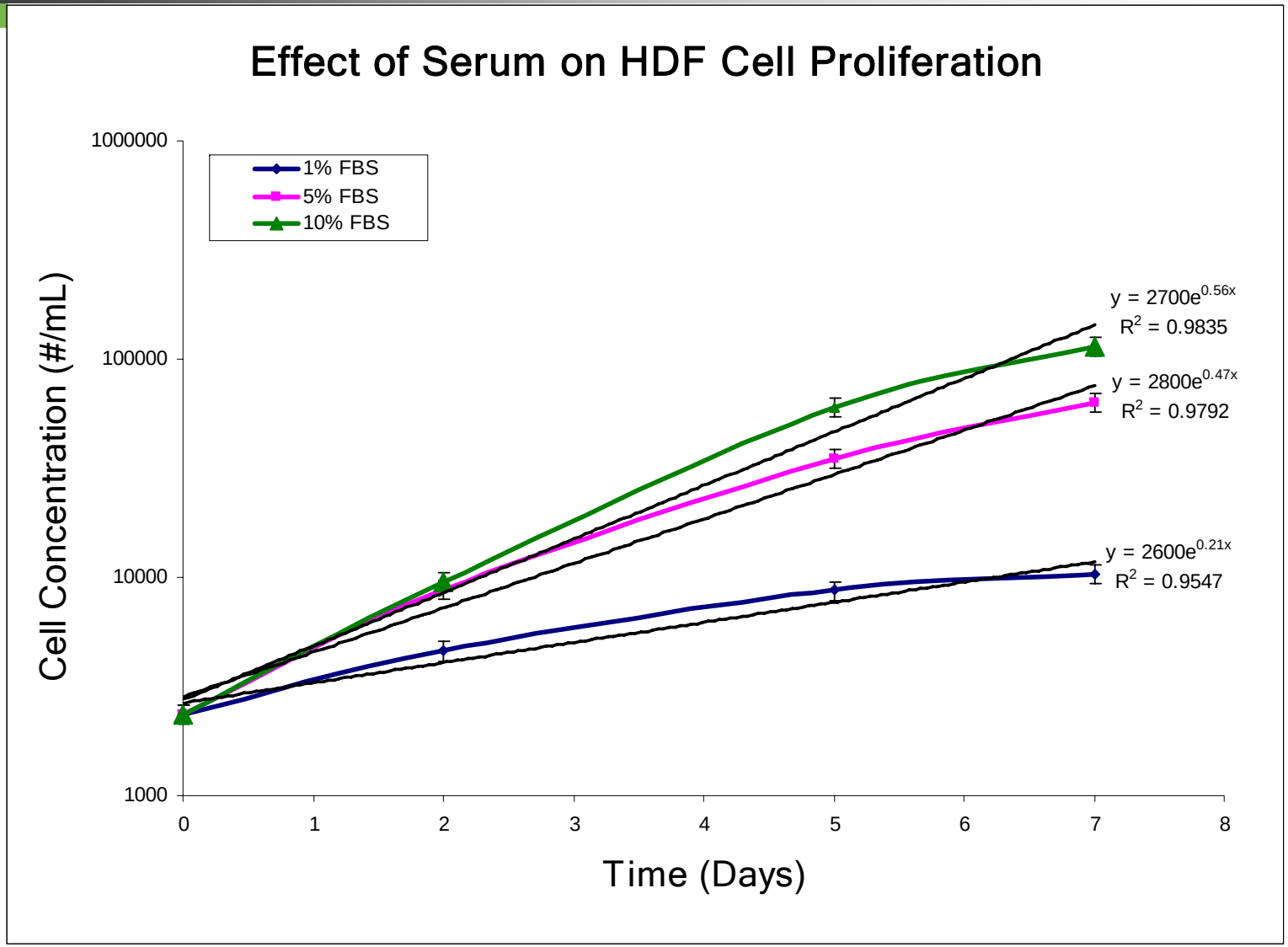
Assessing HDF Cell Viability

- MTT Viability and Live/Dead assays both quantify metabolically active, live cells
- Each assay individually contains drawbacks
  - Cell count with Coulter Counter includes dead cells and cell debris
  - Live/Dead qualitative, does not yield precise cell count
- Obtain most accurate cell count when used together
  - Dye live cells and count with hemocytometer
  - Quantify live cells with Live/Dead assay, then run MTT Viability assay with adjustments from Live/Dead to remove effects of dead cells and debris

HDF Cell Proliferation Assay Experimental Procedure

- Day 0 – Plate cells
- Add DMEM with 1%, 5%, and 10% FBS to samples for Days 2, 5, and 7.
- Determine cell concentration at Days 0, 2, 5, and 7.

HDF Cell Growth is Exponential



HDF Proliferation Varies with Serum Conditions

- Significantly greater cell number in 10% serum on Day 7 than Days 2 and 5 ($p=1.7\times10^{-6}$, Anova and Tukey's)
- Significantly greater cell number on Day 7 in 10% serum than in 1% and 5% serum ($p=1.1\times10^{-6}$, Anova and Tukey's)

% Serum	Doubling Time (hours)
1	79
5	35
10	29

- Cell doubling time decreases with increased % FBS
- 10% serum optimal out of 3 tested DMEM conditions for maximum cell proliferation

HDF Viability and Proliferation: Additional Experiments

- Use Live/Dead assay to assess effects of exponential cell growth on cell viability and mortality

- Use MTT Viability assay to obtain more accurate cell counts from cell proliferation assay; assess effects of exponential cell growth on number of metabolically active cells