Proliferation and Viability of Human Dermal Fibroblast Cells In Vitro

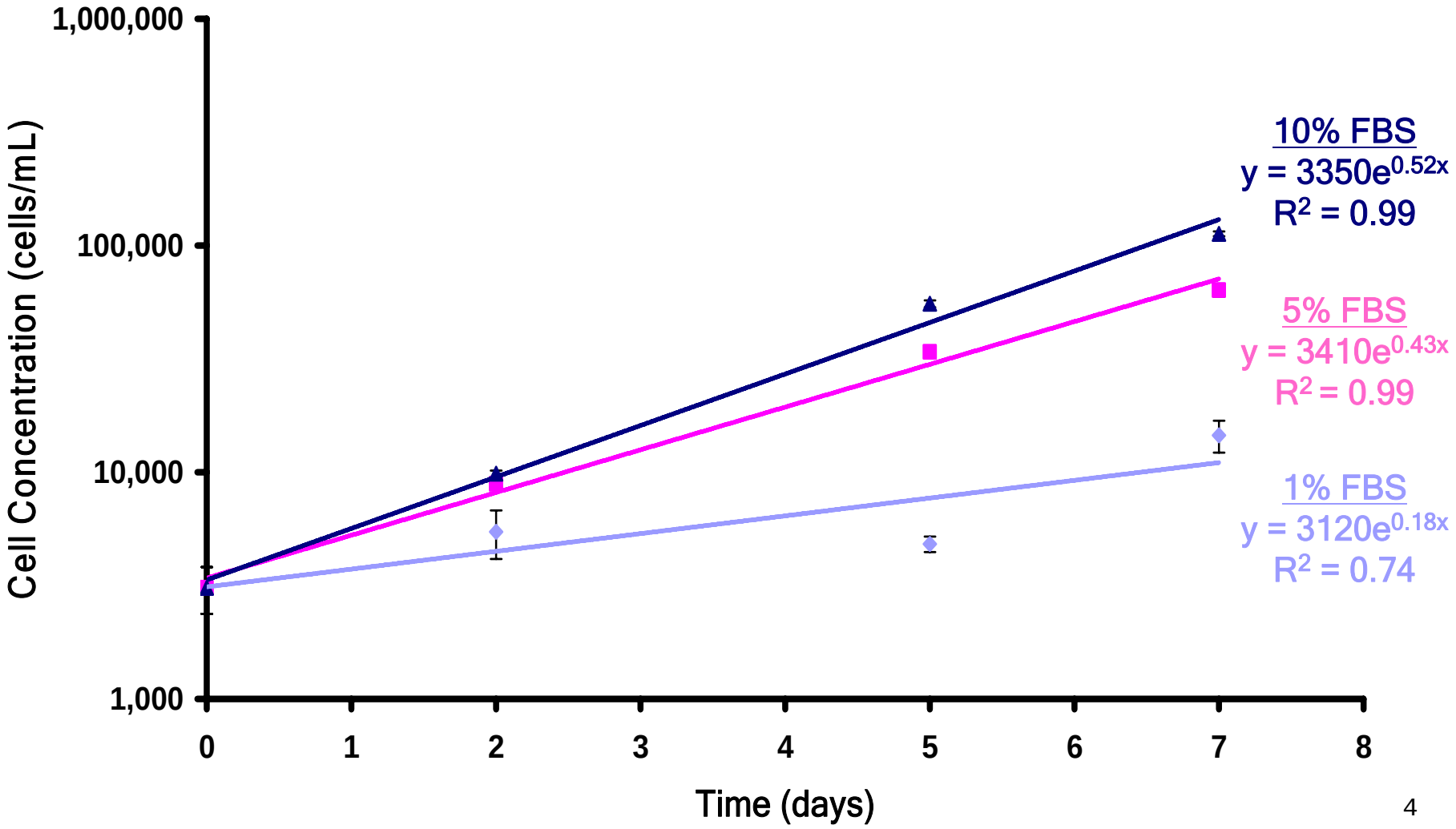
Experimental Objectives

- To quantitatively assess cell replication and growth in response to serum concentration
  - Cell Proliferation Assay
- To qualitatively observe effect of toxic substance on cells
  - Live/Dead Fluorescence Assay
- To analyze relationship between absorbance and cell number
  - MTT Viability Assay

Proliferation Assay Methods

- **Seeded 5,000 cells/well in DMEM with 1% Fetal Bovine Serum (FBS)**
- **After 4 hour incubation:**
  - Verified cell attachment with light microscope
  - Determined cell concentration using Coulter Counter
  - Changed media to 1%, 5%, or 10% FBS in DMEM
- **On days 2, 5, and 7:**
  - Observed cell confluency with light microscope
  - Determined cell concentration for each condition using Coulter Counter
  - Replenished media as needed

HDF Cell Growth Exponential in 5% and 10% FBS



FBS Significantly Affects Cell Proliferation

- **Significant difference between cell concentrations:**
  - At days 0, 2, 5, and 7 within each condition (ANOVA, $p<.005$)
    - → Cells proliferate over time in all conditions.
  - At day 7 for each condition (ANOVA, $p<.005$)
    - → Greatest observed proliferation in 10% FBS
    - → Serum concentration and growth rate positively correlated
- **Doubling time (T_D) decreases with increase in FBS concentration**

% FBS	1	5	10
T_D (days)	3.9	1.6	1.3

Live/Dead Fluorescence Assay Methods

- Cells seeded in DMEM with 10% FBS and incubated 2 days
- Well treatments:
  - PBS + Dye
  - Ethanol + Dye
  - PBS + 2 Drops Ethanol + Dye
- Morphology of cells examined under light microscope
- Cell viability assessed with fluorescent microscope:
  - Calcein AM stains live cells green
  - Ethidium homodimer stains nuclei of dead cells red

Microscopy Reveals Cytotoxicity of Ethanol

Condition	Light Microscope Observations of Cell Morphology	Fluorescent Microscope Observations	Conclusion
PBS	Attached and elongated	Green cells	PBS not cytotoxic
Ethanol	More rounded and partially unattached	Red nuclei	Cells killed upon exposure to ethanol
PBS + Ethanol	Rounded, unattached and elongated, attached	Green cells and red nuclei	Decrease in ethanol cytotoxicity with decreased concentration

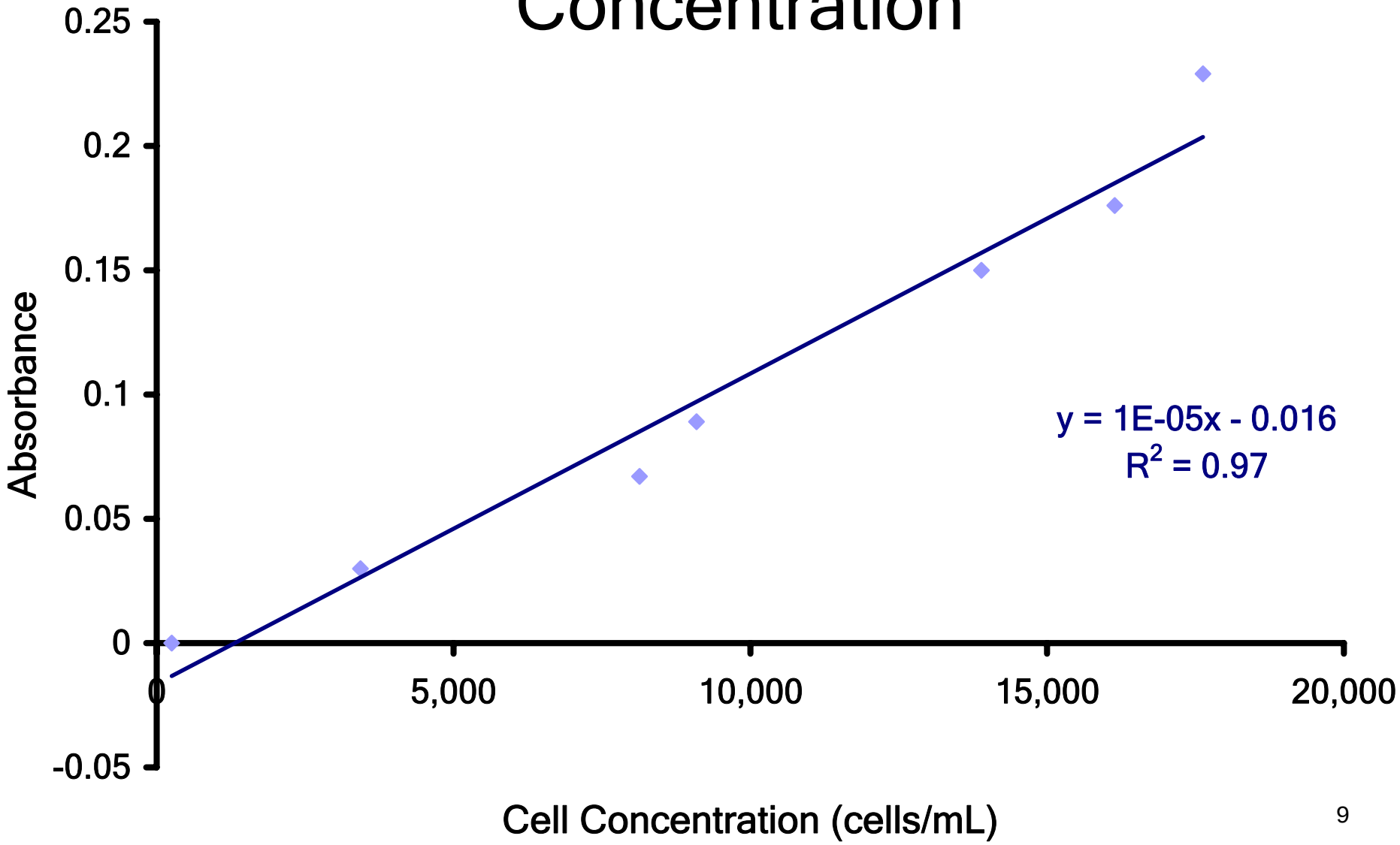
MTT Assay Methods

- Seeded cells in DMEM with 10% FBS:

Well	1	2	3	4	5	6	7
Concentration (cells/mL)	50,000 (stock)	33,500	25,000	16,700	8,330	4,170	0 (only media)

- Verified cell concentration with Coulter Counter
- Incubated cells with MTT Dye and Solubilization/Stop Solution
  - Metabolizing cells reduce MTT dye
- Absorbance at 570 nm, determined by spectrophotometer, quantifies reduced product

Absorbance Relates Linearly to Cell Concentration



Comparison of Viability Assays

- **Live/Dead Assay:**
  - Direct viability assessment with dye
  - Dead and live cells clearly distinguished by fluorescence
- **MTT Assay:**
  - Indirect viability assessment with dye
  - Absorbance of metabolizing cells a function of cell concentration
    - Cell concentration determined with Coulter Counter (indiscriminate to cell viability)
    - No dead cells in PBS condition of Live/Dead Assay, so linear relationship accurate
  - If cells killed with ethanol, no absorbance
- **Coulter Counter:**
  - Disadvantage: cells exposed to ethanol counted

Conclusions

- **FBS promotes HDF proliferation**
  - Cell growth exponential for 5% and 10% FBS
  - 10% FBS presents shortest doubling time because contains more growth-promoting factors
    - A time-saver if need to grow cells quickly
- **Live/Dead Assay stains live cells green and nuclei of dead cells red**
  - Ethanol cytotoxic
  - PBS nontoxic to cells
- **Linear trend between absorbance and cell concentration**
  - Spectrophotometry a valuable alternative technique to measure cell concentration