HDF Viability and Proliferation in Vitro

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Purpose

- **MTT Assay Test**
  To calculate the relationship between cell concentration and MTT dye absorbance (at 570 nm)
- **Anti-PCNA Staining**
  To observe the effects of different media conditions on the rate of cell division
- **Cell Proliferation Assay**
  To observe cell proliferation in cultures incubated in media with varying serum concentrations (1%, 5%, 10%)

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Measuring MTT Dye Absorbance at Different Cell Concentrations

- Seeded two sets of human dermal fibroblast (HDF) solutions at 7 different concentrations ranging from 0 to 50,000 cells/mL and incubated for 48 hours.
- Measured cell concentration for one set using Coulter Counter.
- Treated second set with MTT dye and measured absorbance at 570 nm using spectrophotometer.
- Graphically derived relationship between absorbance levels and cell concentration.

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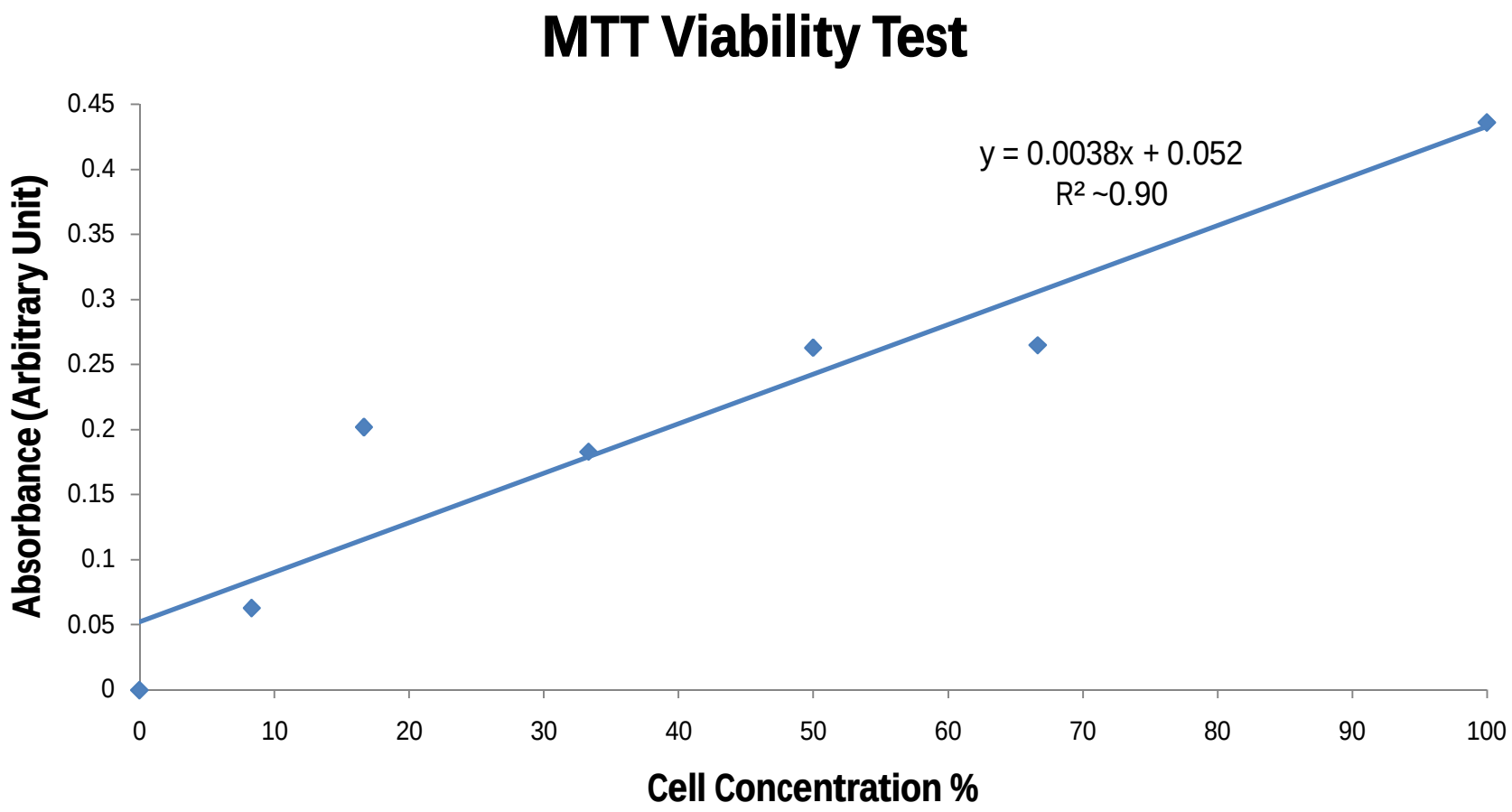
Linear Relationship Between Concentration of Viable Cells and Absorbance



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Absorbance Increases Linearly with Cell Concentration

- Wavelength setting of spectrophotometer (570nm) corresponds to absorbance of metabolized MTT dye, which is purple.
- Therefore, measured absorbance proportional to viable (metabolizing) cell concentration.

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Observing the Number of Dividing Cells Using Anti-PCNA Staining

- Seeded HDF cells (20,000 cells/mL) in DMEM with 1%, 5%, and 10% serum; incubated cells for 48 hours.
- Treated cells with primary antibody that reacts with PCNA protein and then with Horseradish Peroxidase (HRP) secondary antibody, which reacts with staining agents Aminoethyl carbazole (AEC) and hematoxylin.
    - AEC stains nucleus of dividing cells red
    - Hematoxylin stains nuclei of all cells blue
- Used light microscope to observe and estimate percentage of cells in S-phase (stained red) under each media condition.

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Cells Undergoing Division Indicated by Anti-PCNA Staining

% Serum in Media	Fraction of Red Stained Nuclei
10	67 %
5	40%
1	15%

- Red stain marked nuclei of cells in S-phase and actively growing.
- Greater percentage of cells in media with higher serum concentration more frequently exhibited red stain.

* Data from XXXXXX

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Increased Cell Viability at Higher Serum Concentrations

- Higher concentration of serum provides increased availability of nutrients required for cell growth and division.
- Consequently, higher concentrations of serum promote a greater number of cells to actively undergo mitosis.
- This also results in a greater number of cells in S-phase at a given time and is reflected in the Anti-PCNA staining outcome.

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Measuring Proliferation of Cells Incubated in Varying Serum Content

- Seeded HDF cells onto TC-treated plate in DMEM with 1% serum and incubated for 4 hours.
- Measured cell concentration of HDF cell suspension using Coulter Counter (Day 0).
- Replaced media of wells with 3 separate conditions of media: DMEM with 1%, 5%, 10 % Serum (FBS).
- At each time benchmark (Day 2, Day 5, Day 7), treated wells with trypsin and measured cell concentration for each media condition using Coulter Counter.

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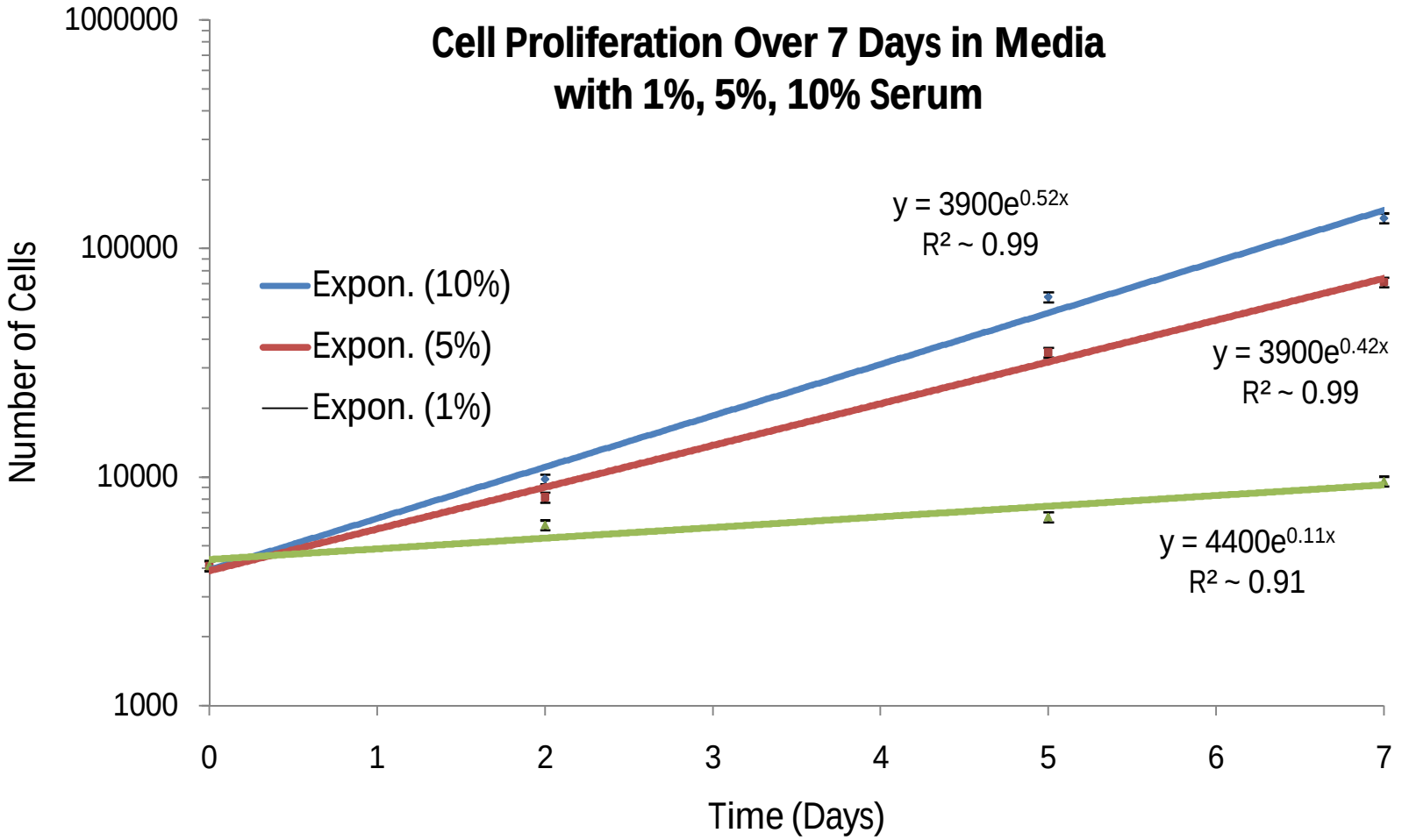
HDF Cells Exhibit Exponential Growth Rate



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HDF Cells Proliferate Exponentially at Varying Rates

- Cells proliferate at a greater rate in media with higher serum (FBS) concentration.
- The differences in rate were observed graphically and further evaluated by calculating by doubling time for each condition.

Concentration of FBS	Doubling Time (days)
10%	1.7 days
5%	2 days
1%	4.6 days

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Anti-PCNA and Proliferation Assay Reveal Common Trend for FBS

- Anti-PCNA staining qualitatively demonstrated that higher percentage of FBS in media results in larger fraction of cells in S-phase.
- Cell proliferation assay quantitatively revealed that higher percentage of FBS in media resulted in greater number of cells.
- Understanding the connection of S-phase to the growth cycle of cell, it can be deduced that Anti-PCNA staining and the cell proliferation assay demonstrate similar conclusions.

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Summary: HDF Viability and Proliferation

- Cell concentration and absorption of metabolized MTT dye share a linear relationship.
- Among the observed media conditions (DMEM with 1%, 5%,10% FBS), higher serum concentration yielded a greater percentage of cells in S-phase and greater proliferation rate of cells overall.

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