Human Dermal Fibroblast (HDF) Characterization *in vitro*

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BIOE 342

Objectives

- Measure cell concentration using absorbance
  - MTT Viability Test
- Observe effects of media conditions on cell growth and replication
  - Anti-PCNA Staining
  - Cell Proliferation Assay

Methods: *Cell Viability*

- MTT Viability Test
  - Cells seeding
    - 7 cell concentrations ranging from 0 to 50,000 cells/mL
    - Identical seeding on second plate
    - Both plates incubated for 2 days
  - Samples in first plate trypsinized
    - Number of cells in each well counted using Coulter Counter
  - Samples in second plate treated with MTT dye
    - Absorbance of each sample measured with spectrophotometer

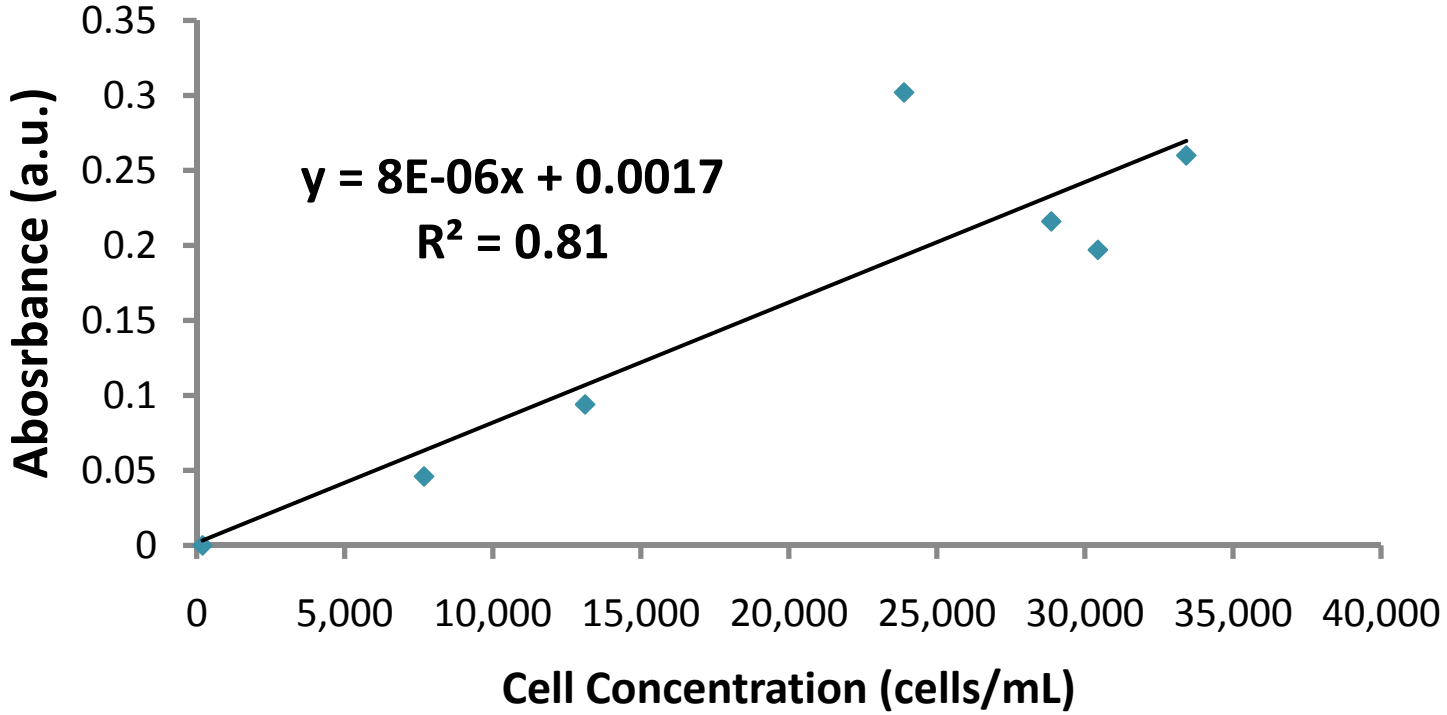
Methods: *Cell Replication*

- Anti-PCNA Staining
  - Cell seeding
    - 20,000 cells/mL
    - 1%, 5%, or 10% fetal bovine serum (FBS)
    - Incubated for 2 days
  - Staining
    - Anti-PCNA primary antibody added, followed by Anti-mouse IgG secondary antibody
    - Cells stained with AEC chromagen and hemotoxylin
  - Light Microscopy
    - Cell nuclei in S-phase stained red
    - Cell nuclei not in S-phase stained blue

Methods: *Cell Replication (cont'd)*

- Cell Proliferation Assay
  - Cell seeding
    - 5,000 cells/mL in 1% FBS
    - After 4 hours, media replaced with either 1%, 5%, or 10% FBS
    - Incubated for 4 hours, 2 days, 5 days, or 7 days
  - Samples trypsinized at assigned time point
    - Cell number counted for each media condition using Coulter-Counter
    - Media replenished for remaining time point samples

Cell Concentration and Absorbance Positively Correlated



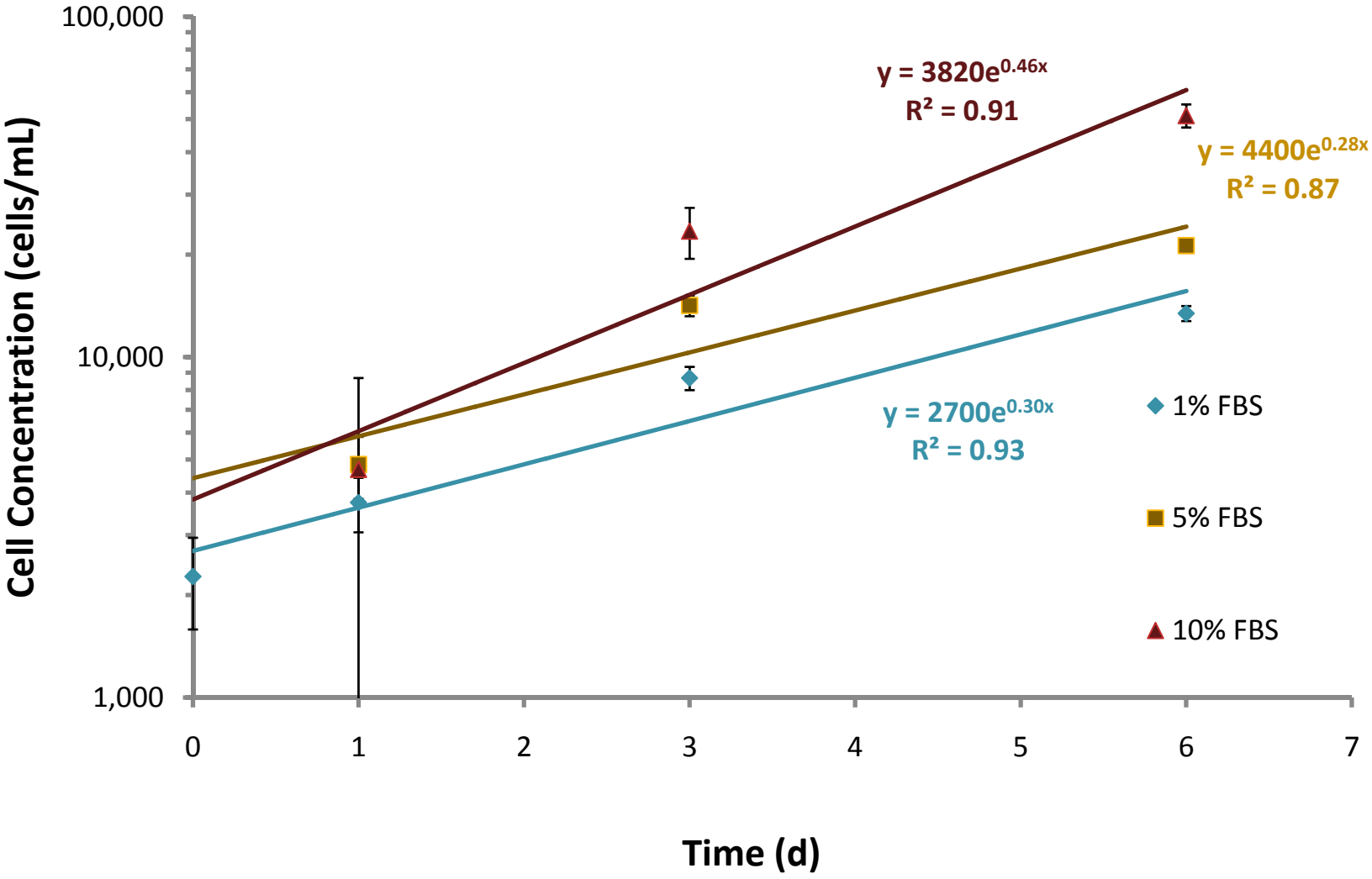
- The formula derived from the linear relationship can be used to approximate HDF concentration in a sample if its absorbance is known.

Higher Serum Concentrations Promote Percentage of Cells in S-Phase

FBS Conc.	1%	5%	10%
% of cells in S-Phase	35	70	90

- As concentrations of FBS in media increased, more cell nuclei were stained red, indicating they were in S-Phase.
- Cells in S-phase are replicating their DNA in preparation for mitosis.

Higher Serum Concentrations Promotes Cell Proliferation



FBS Concentration affects Cell Replication

- After 6 days, cells cultured in 1%, 5%, and 10% FBS had significantly different concentrations (ANOVA with Tukey's HSD, $p<.001$)

FBS Conc.	1%	5%	10%
Doubling Time (days)	2.37	2.44	1.50

- Cells cultured in 10% FBS replicated 1.6 times faster than those cultured in 1% FBS.

Correlation of Number of Cells in S-Phase with Proliferation

- With higher FBS concentrations:
  - From Anti-PCNA:
    - More cells are in S-Phase
      - Commitment to cell division
    - No information about rate of division
  - From Proliferation Assay:
    - Doubling time decreases
    - Cell concentration increases
    - No information about cell cycle activity
- Cell population growth is dependent on the availability of nutrients found in serum.

Conclusions

- A linear relationship exists between cell concentration and absorbance.
- Serum concentration in media affects:
  - Number of cells in S-Phase
  - Proliferation
  - Growth Rate
- Cells in S-Phase are indicators of proliferation.
  - DNA replication indicates cells are in the process of dividing.
- Nutrient availability limits the number of cells undergoing replication as well as the rate at which they replicate.