Human Dermal Fibroblast (HDF) Cell Proliferation and Viability

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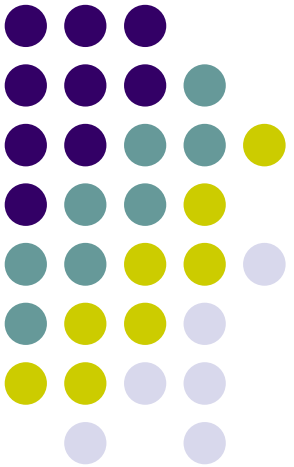


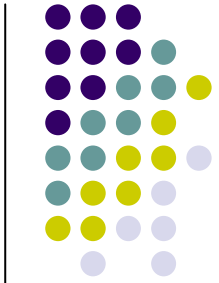
Objectives

- To determine relationship between:
  - Human Dermal Fiboblast (HDF) cell proliferation and 1, 5, and 10% serum
  - HDF cell cycle and 1, 5, and 10% serum
- To analyze the effect of different percentages of serums on HDF cell proliferation
- To quantitatively determine relationship between MTT dye absorbance and HDF cell concentration

Anti-PCNA Staining Methods

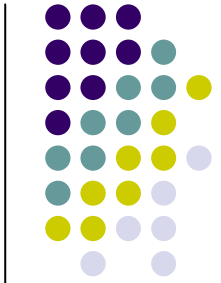
- Seed 3 wells in DMEM with 1,5, and 10% serum
- Add to wells (in order):
  - Anti-PCNA primary antibody
  - Anti-mouse IgG secondary antibody
- Stain cells with:
  - AEC solution
  - Hematoxylin
- Observe with light microscope

Cell Proliferation Assay Methods



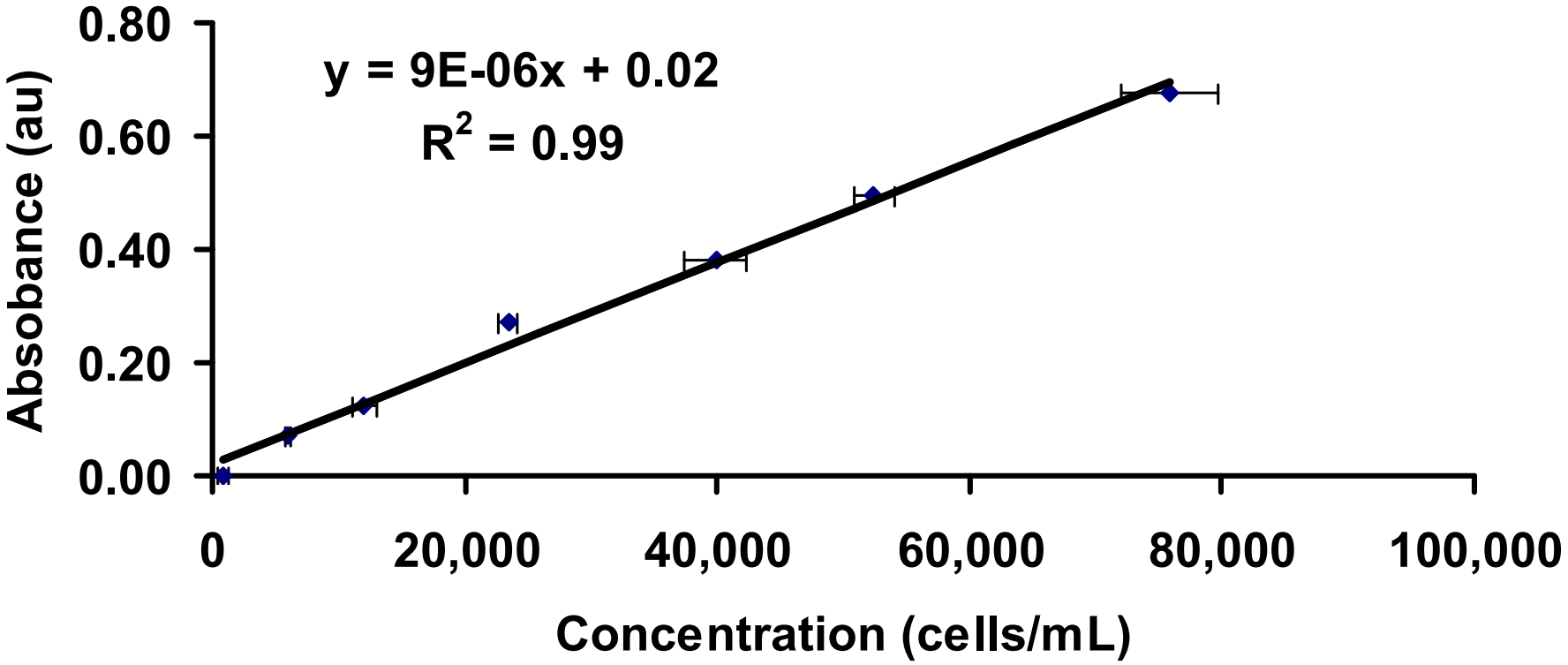
- Seed 27 wells at 5,000 cell/mL with DMEM with 1, 5, and 10% serum (9 wells with each %)
- Use the Coulter Counter to determine cell concentration of each % serum 4 hours, 2, 5, and 7 days after seeding

MTT Viability Methods

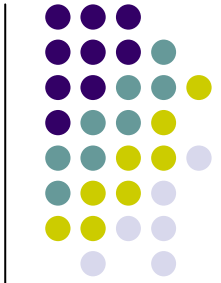


- Seed 6 wells with progressive concentrations and 1 control well with no cells
- Add MTT Dye Solution to all wells and incubate in darkness
- Determine concentration of each well using Coulter Counter
- Determine absorbance for each well using a spectrophotometer

Concentration and Absorbance are Directly Proportional

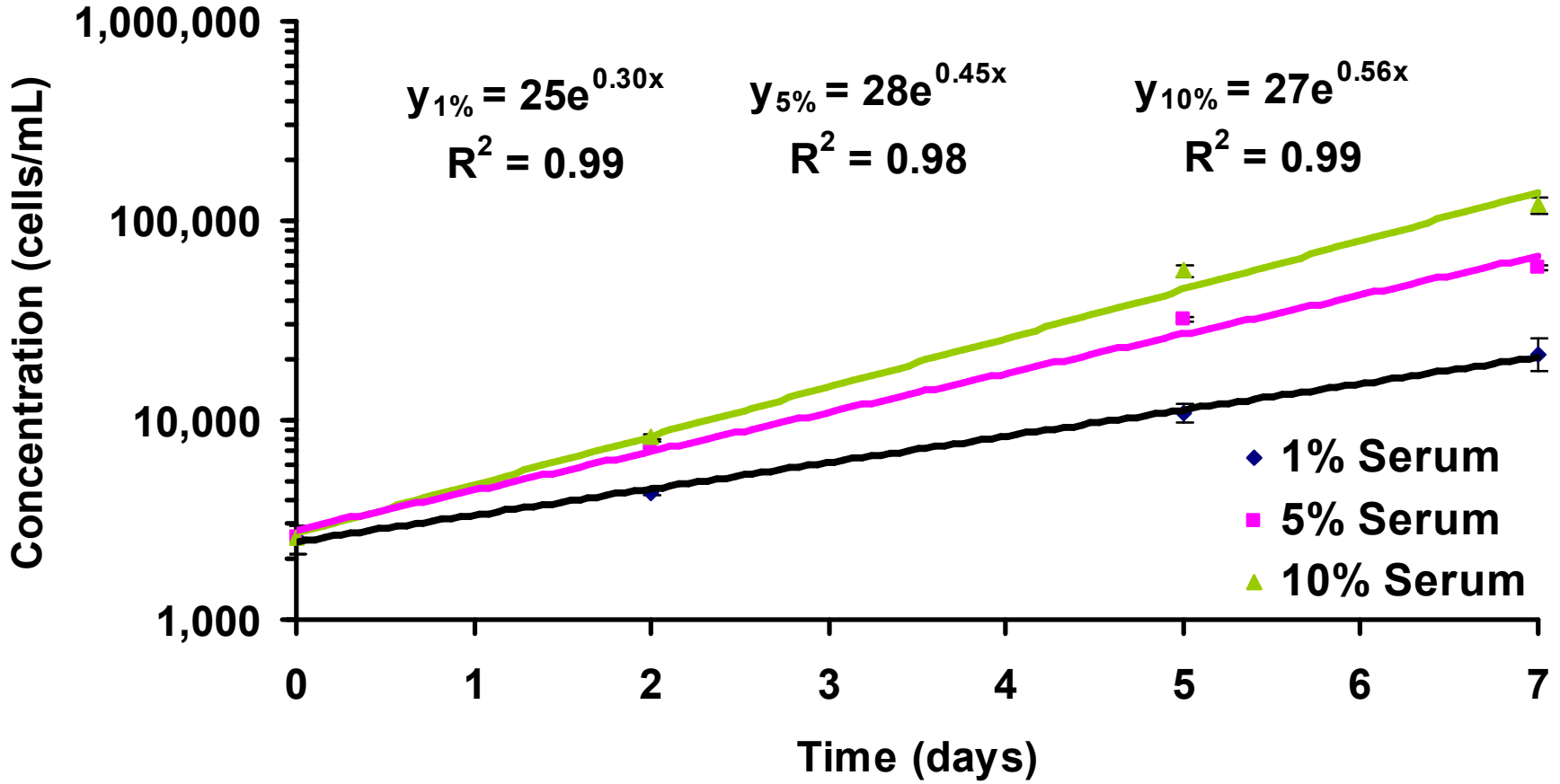


Linear Relationship Between Absorbance and Cell Concentration

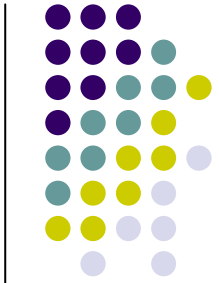


- Graph shows a positive, linear relationship between absorbance and cell concentration
- Statistically, the R^2 value indicates an excellent linear fit to the raw data
- Cell metabolic function turns MTT Dye purple
- Higher absorbance indicates more purple and thus higher cell viability

10% Serum Promotes Greatest Cell Growth



Cells Display Exponential Growth



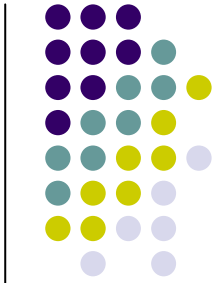
- Graph indicates exponential growth in all three serums
- R^2 values indicate excellent exponential fits for all three serums
- Cells with 10% serum display the largest proliferation
- ANOVA test p-value<.001, indicating a significant difference in final concentrations for all three serums

Anti-PCNA Staining Reveals Cells in S-Phase of Mitosis

% Serum	% Cells with Red Nuclei
1	50
5	80
10	95

- As percentage of serum increases, more cells have red nuclei
- Reveals cells that are proliferating by reddening nuclei fixed in s-phase
- All cells without red nuclei are stained blue

Anti-PCNA Staining and Cell Proliferation Comparison



- Both Anti-PCNA Staining and Cell Proliferation Assay indicate more cells are proliferating in 10% than in 1 or 5% serum
- Type of measurement:
  - Anti-PCNA: qualitative
  - Cell Proliferation Assay: quantitative
- Relationships:
  - Anti-PCNA: number of cells fixed during S-phase increase with increasing percentage of serum
  - Cell Proliferation: cells grow exponentially

Conclusions

- HDF cell proliferation is exponential in DMEM with 1, 5, and 10% serum
- More HDF cells in DMEM with 10% serum are:
  - In S-phase of mitosis
  - Actively proliferating

  than in 1 or 5% serum
- HDF cell proliferation is greater in DMEM with 10% serum than with 1 or 5% serum
- Relationship between absorbance and HDF cell concentration is positive and linear in MTT Viability Assay