Proliferation and Viability of Human Dermal Fibroblasts

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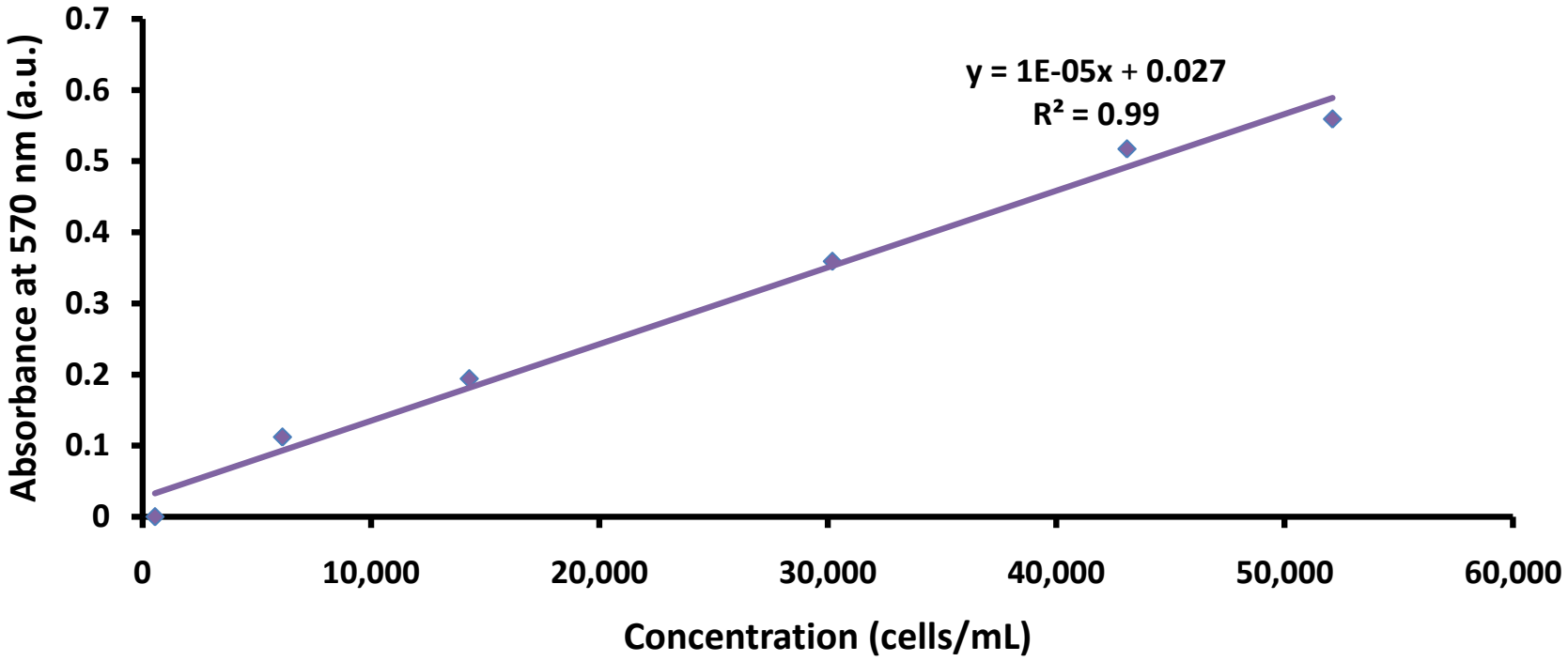
Objectives

- MTT Viability Test
  - To find a linear relationship between absorbance from live cells dyed with MTT and total cell concentration
- Cell Proliferation
  - To assess the effects of serum on growth and replication of HDF cells quantitatively
- Anti-PCNA Staining
  - To use antibody-specific staining to observe the connection between cell proliferation and media conditions

MTT Viability Test Methods

- Seeded cells at dilutions from 50,000 cells/mL to 4,200 cells/mL in two plates
  - Plate for MTT on which the cells would react with the dye and have their absorbance measured using a spectrophotometer
  - Plate that would be counted using a Coulter Counter
- MTT dye reacts with metabolically active cells

Absorbance is Directly Related to Cell Concentration



- Absorbance due to the reaction of the dye and metabolically active cells shows a linearly correlation to total cell concentration

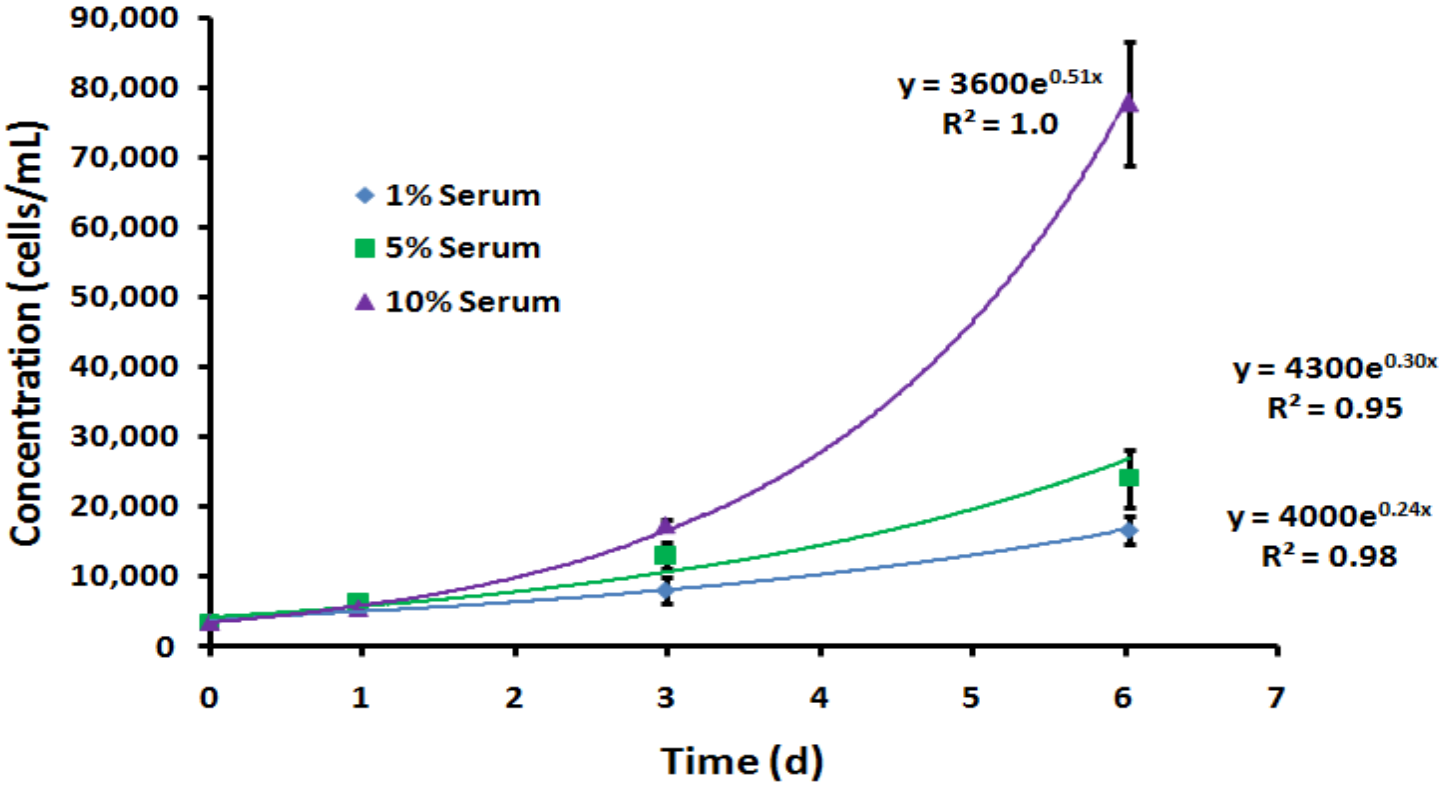
Discussion of MTT Data

- Absorbance is proportional to the concentration of live cells
  - Only metabolically active cells react with dye
- Concentration of live cells is proportional to the total concentration of cells
  - Death rate should be equal in the different dilutions
- Results in the linear relationship between the total concentration of cells and the absorbance

Cell Proliferation Methods

- Cells were seeded at 5,000 cells/mL then had their media changed to DMEM with 1%, 5% or 10% serum for testing
- At 1, 3 and 6 days, three wells of each serum concentration were counted using a Coulter Counter to determine cell concentration
- Media was replenished on day 3 for the day 6 cells

10% Serum Media Increases Cell Proliferation Over 1% and 5% Serum



- Cell grew exponentially in DMEM with 1%, 5% and 10% serum

Discussion of Cell Proliferation Data

- DMEM with 10% serum resulted in the lowest doubling time and fastest growth of the cells of the serum concentrations tests
  - $t_{d,\ 1\%}$= 2.7 days
  - $t_{d,\ 5\%}$= 2.2 days
  - $t_{d,\ 10\%}$= 1.4 days
- DMEM with 10% serum allowed for the most replication, possibly because it increased the amount of nutrients
  - Allows for more cells to divide without having to compete for the nutrients
- On day 6, the different serum conditions resulted in statistically different cell concentrations
  - Shows the proliferation rates are statistically different ($p<0.05$)
  - From ANOVA, $p=0.00$
  - Tukey Test showed that 10% serum was significantly different from 1% and 5% serum but that 1% and 5% serum were not significantly different from one another ($p<0.05$)

Anti-PCNA Methods

- Cells were seeded at 20,000 cells/mL in DMEM containing 1%, 5% and 10% serum
- After 2 days, cells were stained
  - Used two antibodies, the second marked with HRP, hematoxylin and AEC
  - Allowed for the differentiation between cells in the S phase and not in the S phase
  - Antibody specific staining visible using a light microscope to estimate cell density

10% Serum Media Increases Cells in S Phase Over 1% and 5% Serum

Serum Content	Red Density (cells/cm^2)	Blue Density (cells/cm^2)	Red Percentage	Blue Percentage
1%	4500	7300	38%	62%
5%	4200	1200	78%	22%
10%	5400	1800	84%	16%

- Red staining means that the cell contains PCNA and is in the S phase
- Blue staining shows that the cell is not in the S phase
- 10% serum resulted in the highest percentage density of red cells, while 1% resulted in the lowest percentage density of red cells

Discussion of Anti-PCNA Staining Data

- The red color shows the cells contained PCNA for the first antibody to react with, allowing the second antibody to react and the AEC to react with the HRP
  - Higher density of red cells in 10% shows that more cells are in the S-phase
- More cells in the S-phase means more cells are proliferating

Cell Proliferation and Anti-PCNA Staining

- Cell Proliferation and Anti-PCNA staining gave similar results
  - DMEM with 10% serum was the best condition tested for the proliferation of HDF cells
    - Cell Proliferation showed this through the cell concentration over time
    - Anti-PCNA showed this through the density of cells in the S phase at a fixed time point
- More cells in the S phase and more overall cells are both achieved by using DMEM with 10% serum when compared to 1% and 5% serum
  - By combining these results, we can see that more cells in the S phase mean a higher number of cells over time