Effects of Varying Cell and Serum Concentrations on Fibroblast Viability and Proliferation

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Objectives

- Develop a relationship between absorbance and cell concentration
  - MTT Viability Test
- Observe the effects of changing media conditions on the proliferation of Human Dermal Fibroblast (HDF) cells
  - Anti-PCNA Staining
  - Cell Proliferation Assay

MTT Viability Test Reveals the Number of Metabolizing Cells

- Cells were seeded at six different concentrations onto two, 24 well plates and incubated for two days
- MTT Dye was added to one plate and the amount of reduced MTT purple product formed by living cells was qualitatively assessed by measuring absorbance of the samples at 570nm using a spectrophotometer
- Cells of the second plate were trypsinized and counted using a Coulter Counter to determine the cell concentration of each test dilution and control well

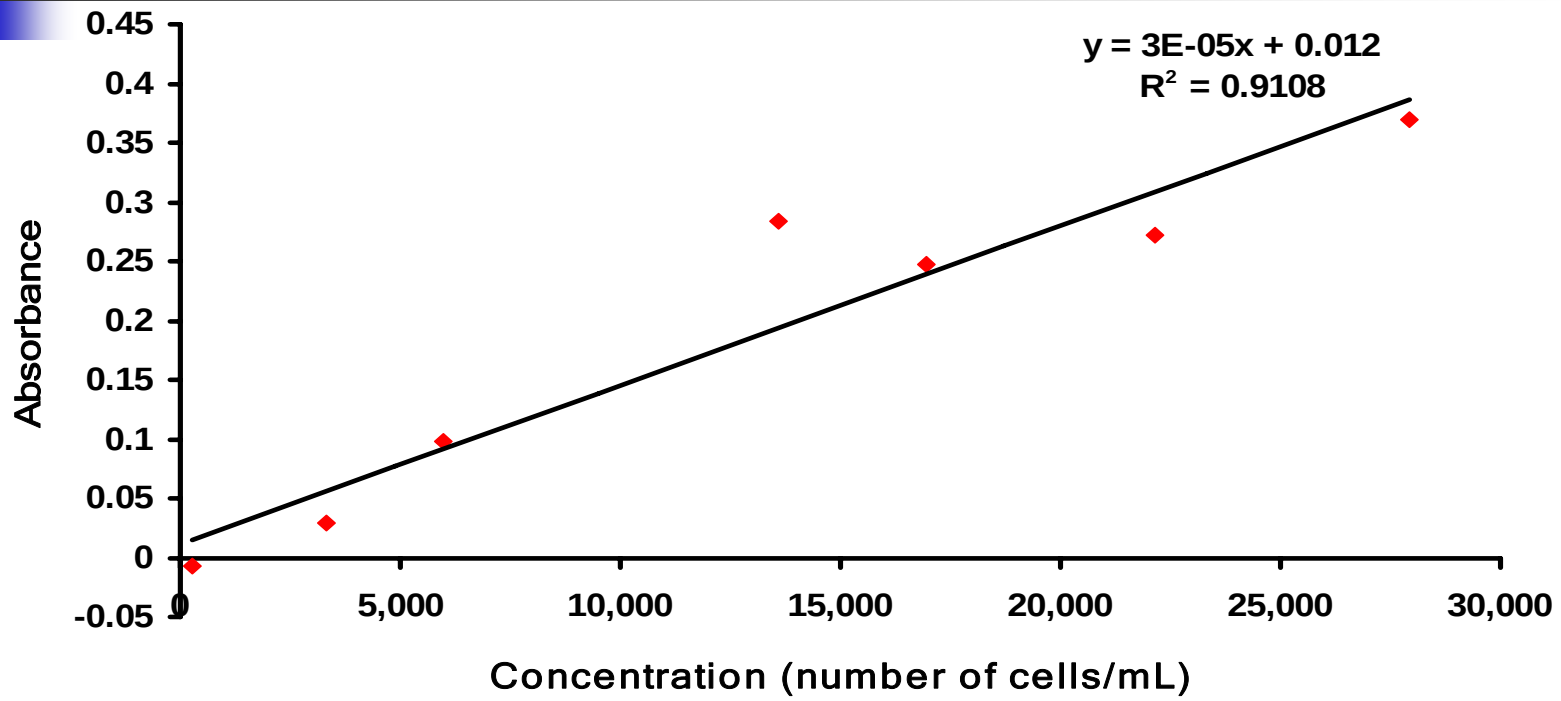
Anti-PCNA labels Cells in S Phase

- Cells were seeded at equal concentrations on 24 well plates and incubated for two days in cDMEM with 1, 5, and 10% FBS (control wells included)
- Horseradish Peroxidase/anti-PCNA was used to stain cell nuclei, in S phase, red
- Hematoxylin and AEC solution was used to stain cells blue
- The percentage of actively dividing cells (nuclei stained violet/red) and non-dividing cells (nuclei stained blue alone) was observed using a light microscope

Measuring Cell Proliferation

- Cells were seeded at equal concentrations on two, 24 well plates and incubated over seven days in cDMEM with either 1, 5, or 10% FBS
- The Coulter Counter was used to determine initial seeding density on Day 0
- On Day 2, 5, and 7 following initial exposure to the different media conditions, cell number for test samples incubating in 1,5, and 10% serum was evaluated using the Coulter Counter
- Media of the varying FBS concentrations was replenished on Days 2 and 5 as needed for the remaining test samples in culture

Absorbance is Linearly Related to Cell Concentration



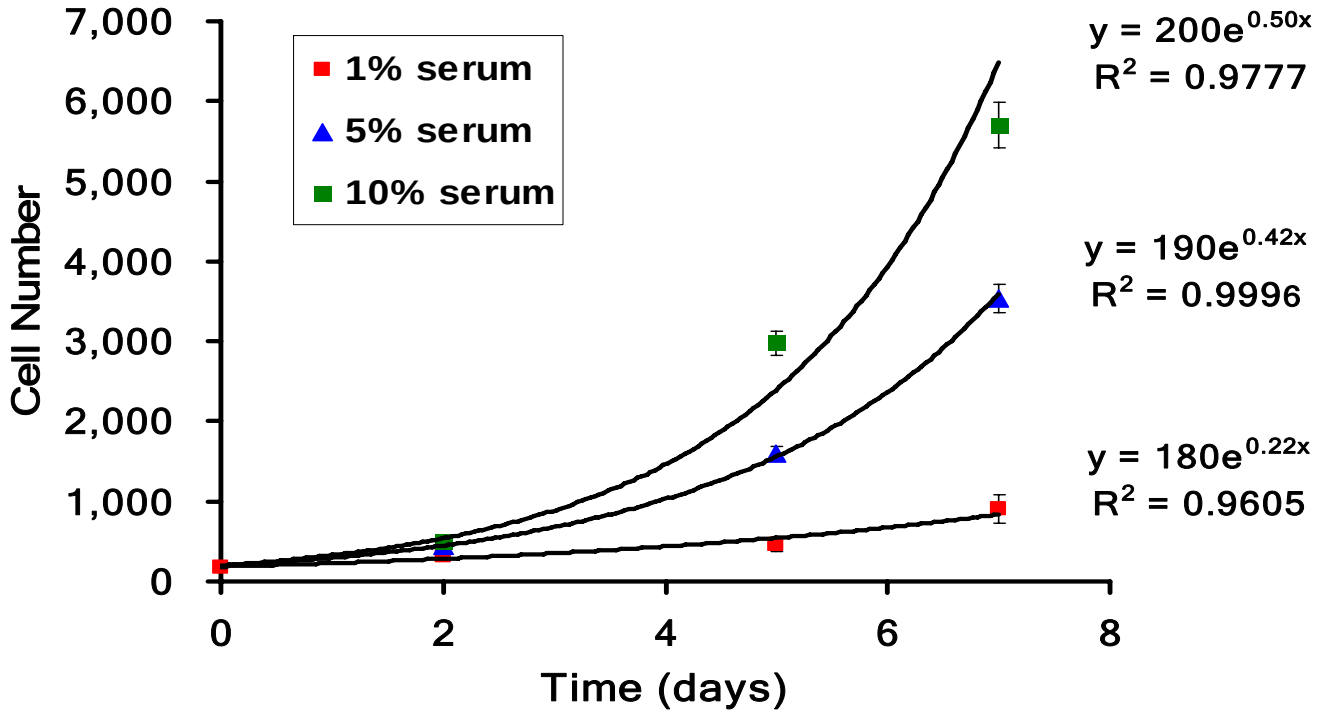
- As cell concentration increases, the absorbance, which is directly proportional to amount of reduced MTT product, also increases due to the greater number of live cells which are metabolizing (n = 7)

Anti-PCNA Staining shows Serum Concentrations Impact Growth Fraction

Test Conditions	% of Actively Dividing Cells	% of Non-dividing Cells
1% FBS + DMEM	30	70
5% FBS + DMEM	40	60
10% FBS + DMEM	60	40

- The growth fraction, or percentage of cells committed to dividing, increases as FBS concentrations increase

Cells Display Exponential Growth



- Cell Numbers at Day 2, 5, and 7 within 10% serum condition are significantly different (n = 3; ANOVA: $p < .05$)
- Number of cells exposed to 1%, 5%, and 10% serum at Day 7 are also significantly different (n = 3; ANOVA: $p < .05$)

HDF Growth Rate Increases as Serum Concentrations Increase

Test Conditions	Cell Doubling Time (hours)
1% FBS + DMEM	93
5% FBS + DMEM	41
10% FBS + DMEM	31

- Data from the cell proliferation assay was used to calculate cell doubling time for each test condition
- As serum concentrations increased, the cell doubling time decreases due to a greater supply of serum components such as growth factors, minerals, and adhesion factors

Proliferation Assays Confirm Growth Fraction is Proportional to Proliferation Rate

- Anti-PCNA staining results showed increasing serum concentrations accompany an increase in the percent of cells in S phase, or actively dividing cells
  - A larger growth fraction indicates there is a greater number of dividing cells and thus, the time to double the cell number of the test sample should decrease
- Proliferation assay results confirm this expected relationship as cell doubling time did in fact decrease with increasing FBS concentrations
  - Shorter cell doubling period yields faster proliferation rates

Conclusions

- The MTT Viability Test uses absorbance readings to measure the metabolic activity of living cells, which is linearly related to cell concentration
  - Future work may entail dye exclusion techniques to determine the concentration of viable cells
- Anti-PCNA staining and the Proliferation Assay reveal HDF growth fraction and proliferation rates increase with greater exposure to the nutrient-rich serum source
  - Further study should be conducted to observe the effects of serum concentrations greater than 10% on HDF proliferation