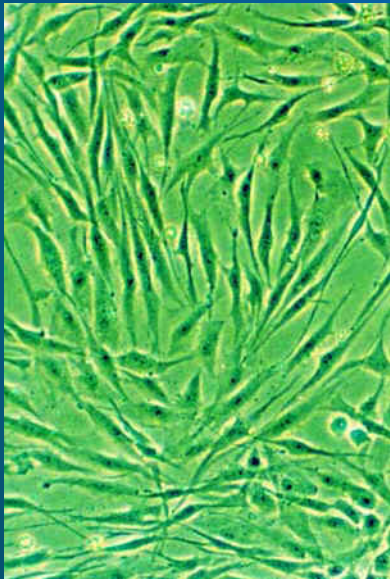
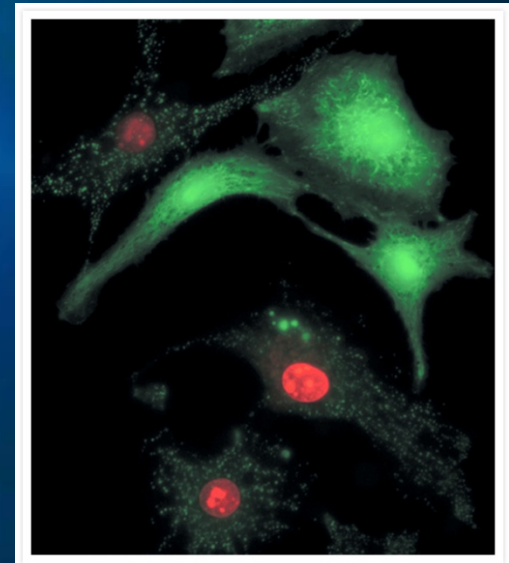


Increasing Serum Concentration Increases HDF Cell Proliferation in a Dose Dependent Manner



YYYY
Bioengineering 342
Section 1



Objective

- Develop a model to describe cell proliferation as a function of time and serum concentration
- Does increasing the concentration of serum in media increase Human Dermal Fibroblast (HDF) proliferation in a **dose dependent** manner?

Quantitative Cell Attachment Assay

- HDF passage 6
- Seeded 12 wells in tissue culture treated and untreated 24 well plates
 - 1 ml at 10,000 cells/ml
- Cells incubated for 30, 75, 150, and 240 minutes
- At each time point
 - Unattached Cells aspirated
 - Attached Cells counted in 10 x 10 grid

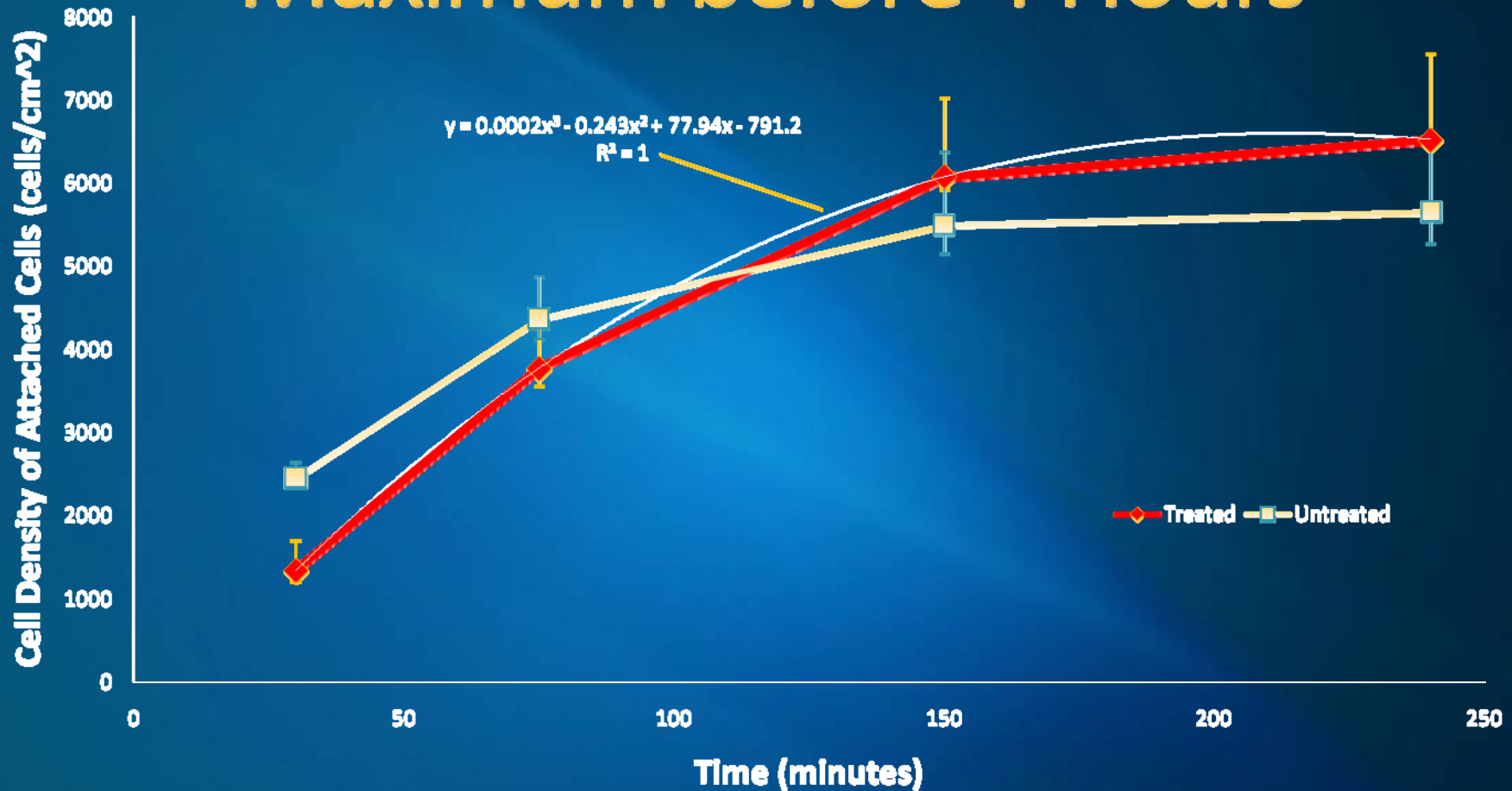
Anti-PCNA Staining

- HDF passage 6
- 1 ml of 1, 5, 10% serum concentration seeded at 20,000 cells/ml
 - 3 additional control wells with 10% serum
- Cells incubated for 2 days
 - 200 μ l of anti-PCNA primary antibody added to test conditions
 - Control 1 – 200 μ l of primary antibody added
 - Control 2 and 3 – 200 μ l blocking buffer added
- 200 μ l of secondary antibody added to test conditions
 - Control 2 – 200 μ l of secondary antibody added
 - Control 1 and 3 – 200 μ l of blocking buffer
- Red and blue dyes added and cells counted under fluorescence microscope

Cell Proliferation Assay

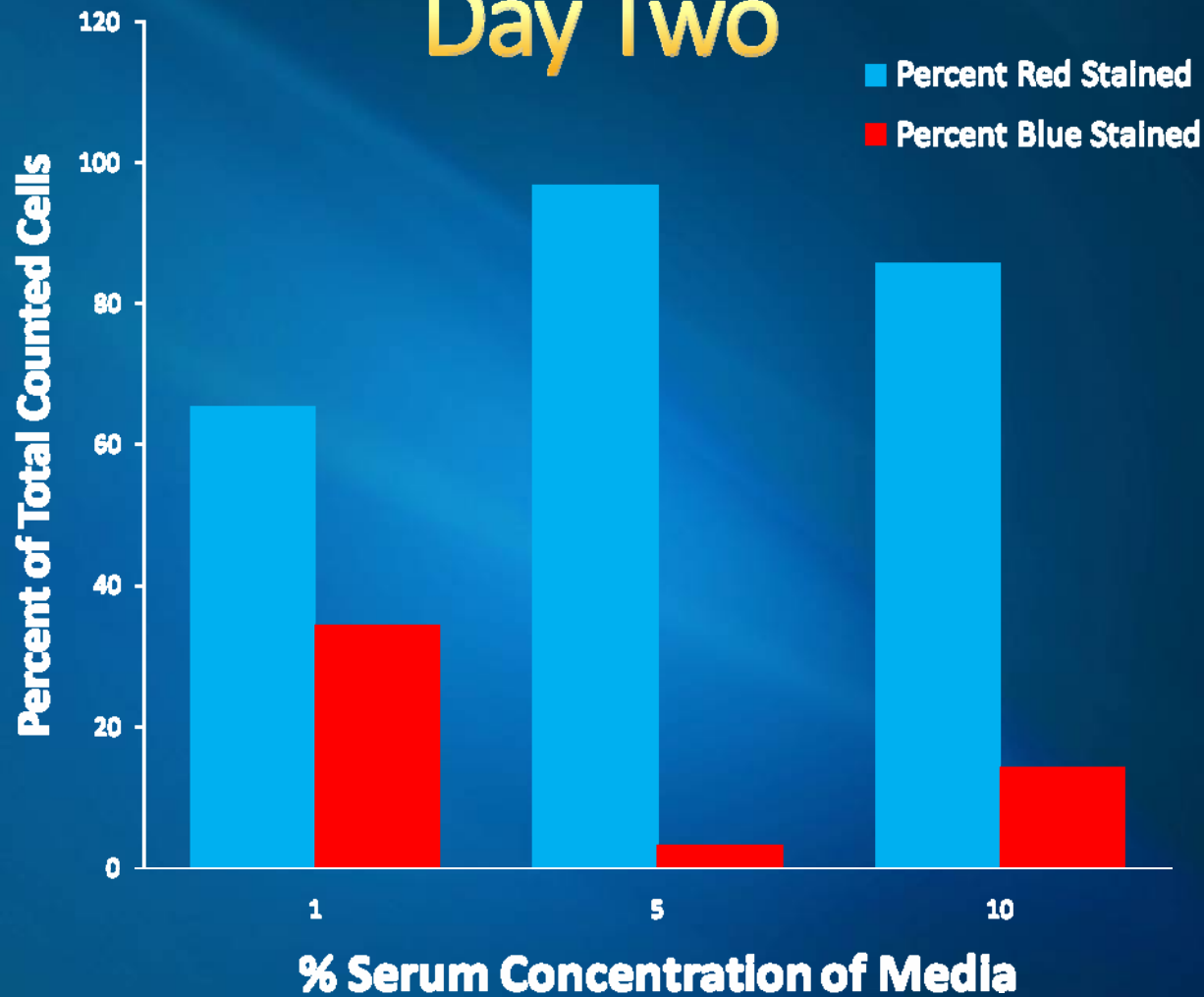
- HDF passage 6
- Cells seeded in 33 total wells
 - 5000 cells/ml
 - 1 % serum concentration
- Cells incubated for 4 hours to ensure attachment
 - Media aspirated
- 1 ml of DMEM with 1, 5, 10% serum added in triplicates for days 2, 5, and 7
- At day 0, 2 , 5, and 7, cells counted with Coulter Counter

Cell Attachment Approaches Maximum before 4 Hours



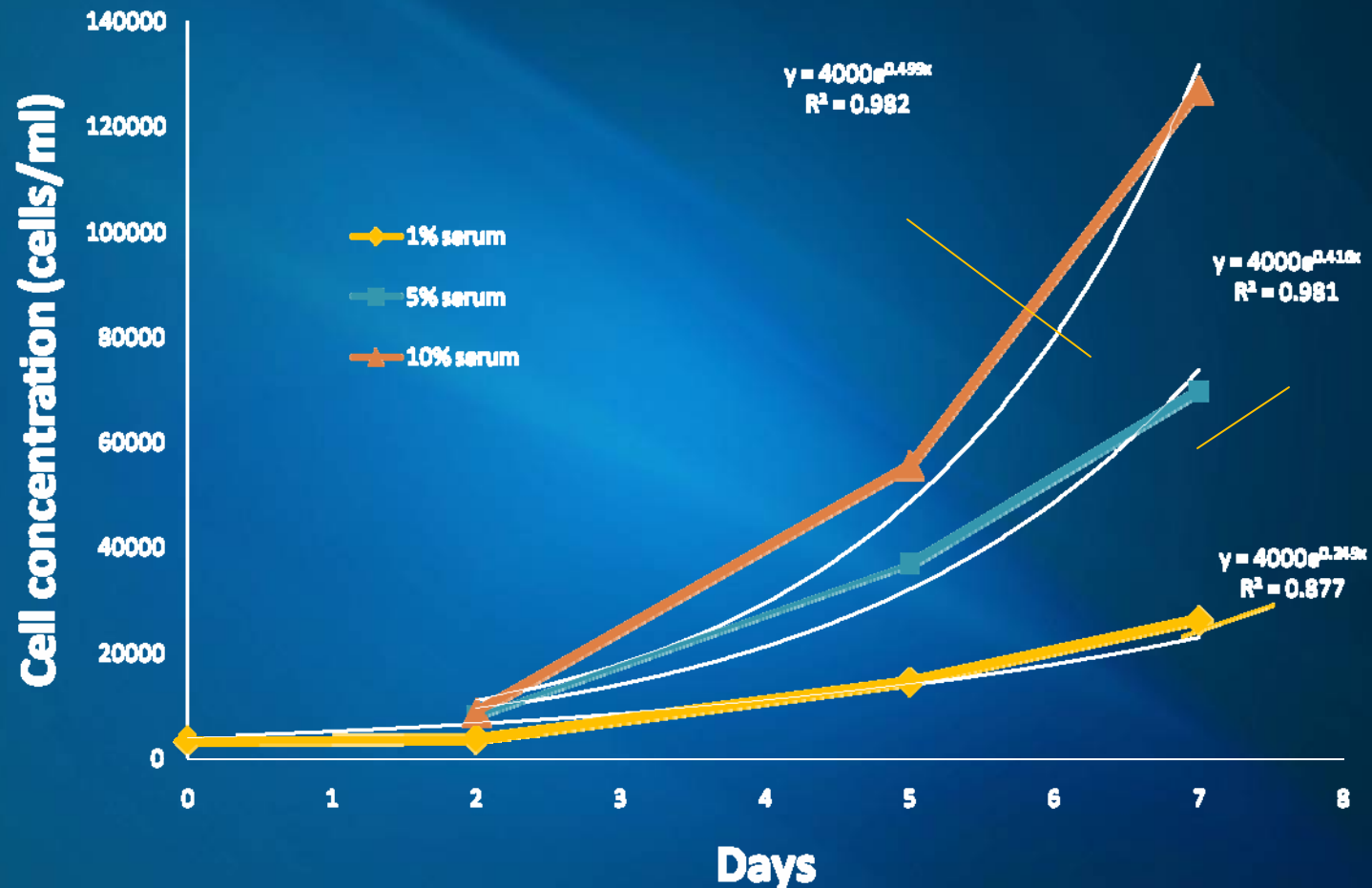
- Using MatLab, maximum occurs at 220 minutes

Cell Proliferation Appears the Same at Day Two



- Total amount of proliferation for 5 and 10% seems the same

Cell Proliferation Increases as Serum Concentration Increases



- ANOVA p value = < .0001
- Cell Proliferation Assay seems to contradict results of PCNA assay

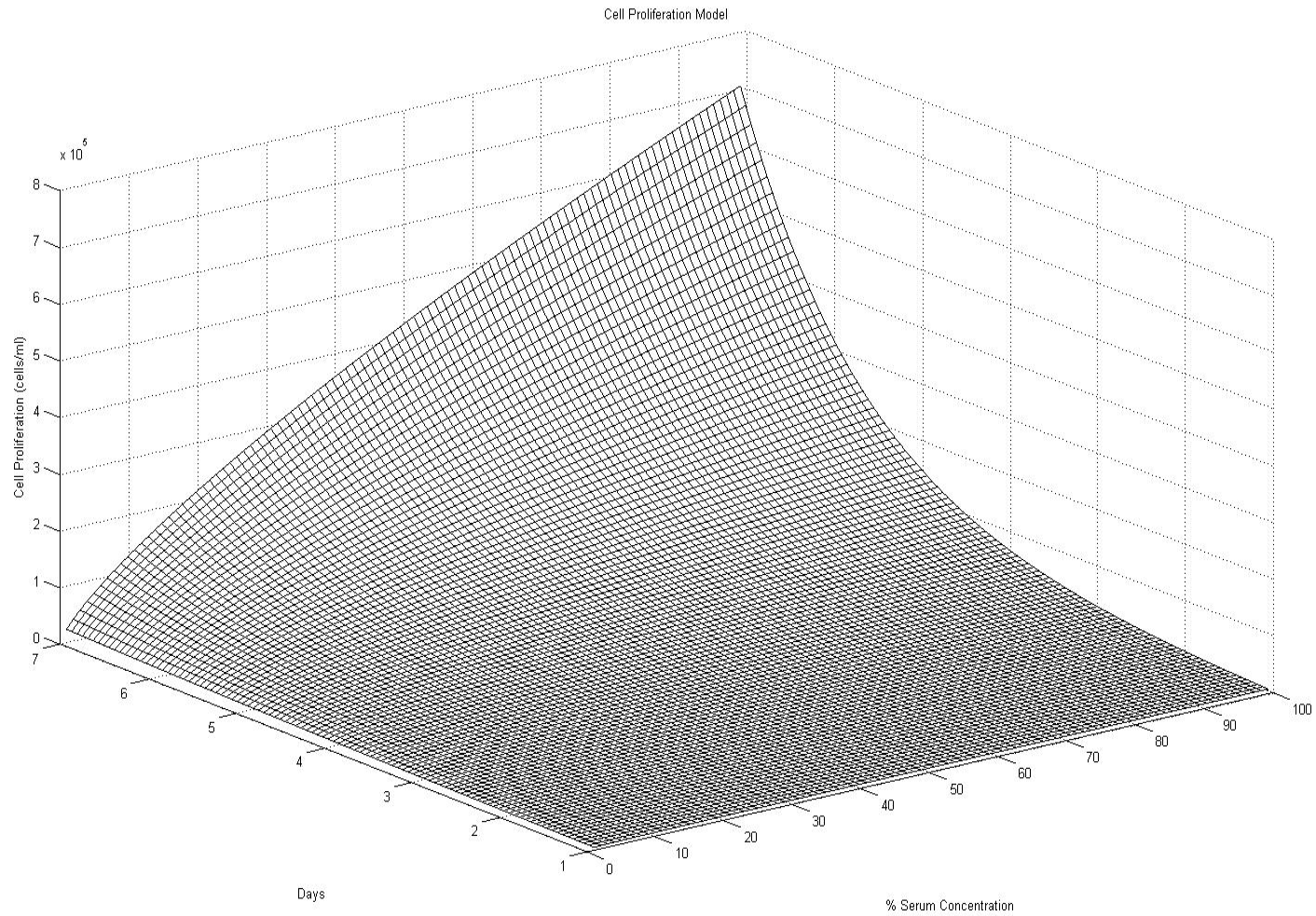
Mathematical Model

- Using MatLab, solved for the relationship between coefficients on x terms and serum concentration in order to create model to predict cell concentration as a function of time and serum concentration
 - Assuming initial concentration of cells, C_0

$$\text{Cell proliferation} = C_0 * e^{((0.107 \ln(\% \text{Serum}) + 0.247) * \text{days})}$$

- True error of model = 1.11%

Cell Proliferation Model



Conclusions

- Quantitative Cell Attachment Assay confirmed that 4 hours is sufficient time in order to ensure that cells were not aspirated out at 4 hour data point during Proliferation Assay
- Anti-PCNA and Cell Proliferation Assay seem to contradict one another
 - Model shows that two days is not enough time to judge a 5% difference in concentration
 - Model predicts a serum concentration of 70% in order to see a 50% increase in cell proliferation
- Cell proliferation increases with serum concentration in a dose dependent manner