

A Quantitative Study of HDF Cell Proliferation and Adhesion

YYY

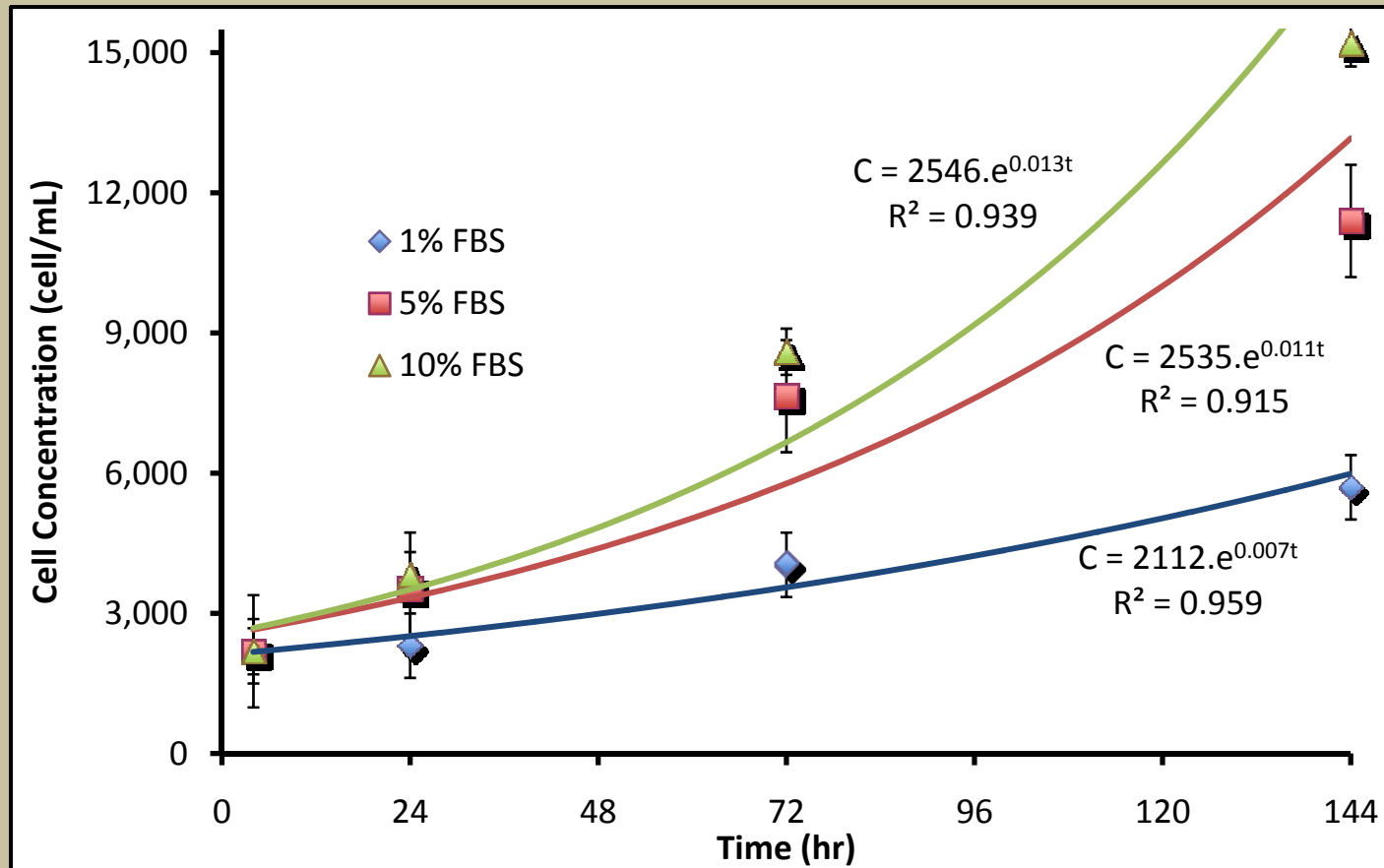
Objectives

- To Determine the dependence of cell proliferation on fetal bovine serum (FBS) content in media
 - Cell Proliferation Assay
- To Analyze the effect of FBS content in media on the percentage of cells in S-phase
 - Anti-PCNA Staining Assay
- To quantify cell attachment over time on various plating surfaces
 - Cell Attachment Assay

Cell Proliferation Assay Methods

- Cells are seeded in 33 wells and incubated in 1% FBS for 4 hours
- Concentration of six wells is determined using Coulter Counter (CC) at hour 4
- Media is aspirated in remaining wells and 1mL media with 1%, 5%, and 10% FBS are each given to 9 wells
- At 24, 72, and 144 hours, cell density of three wells of each condition are measured with CC₂

Exponential Growth of Cells in Varying % FBS



Three cases are unequal with statistically significant difference (ANOVA $P < 0.05$)
From Tukey post hoc, 1% and 5% are significant with $P < 0.05$
10% case does not show significance with $P = 0.124$

Cell Proliferation Results

- All three cases can be modeled with an exponential fit
 - With fresh media and before confluency, HDF cell population increases exponentially over time
- Higher percentages of FBS in media result in greater proliferation rates
- The rate increase from 1% to 5% FBS is greater than the rate increase from 5% to 10% FBS

Anti-PCNA Staining Methods

- Dilute cells to 20,000 cells/mL in 1%, 5%, and 10% FBS
- Seed one well of each condition and incubate for 48 hours
 - Also seed three additional 1% FBS wells as controls
- Expose experiment wells to 1° and 2° antibodies
- Observe cells under white light microscope
- Count cells with and without red stain
 - Red stained nuclei indicates cell is in S-phase
 - Unstained cell indicates cell is not in S-phase

Anti-PCNA Staining Results

Observed Cells in 10X10 Grid of 20X Objective			
% FBS	Stained Cells	Total Cells	% Stained
1	3	13	23.1
5	29	42	69.0
10	28	33	84.8

- Percent of cells in S-phase at a given time increases as the %FBS increases from 1% to 10%
- The percent increase from 1% to 5% FBS is greater than the percent increase from 5% to 10% FBS
 - Congruent with Cell Proliferation Assay

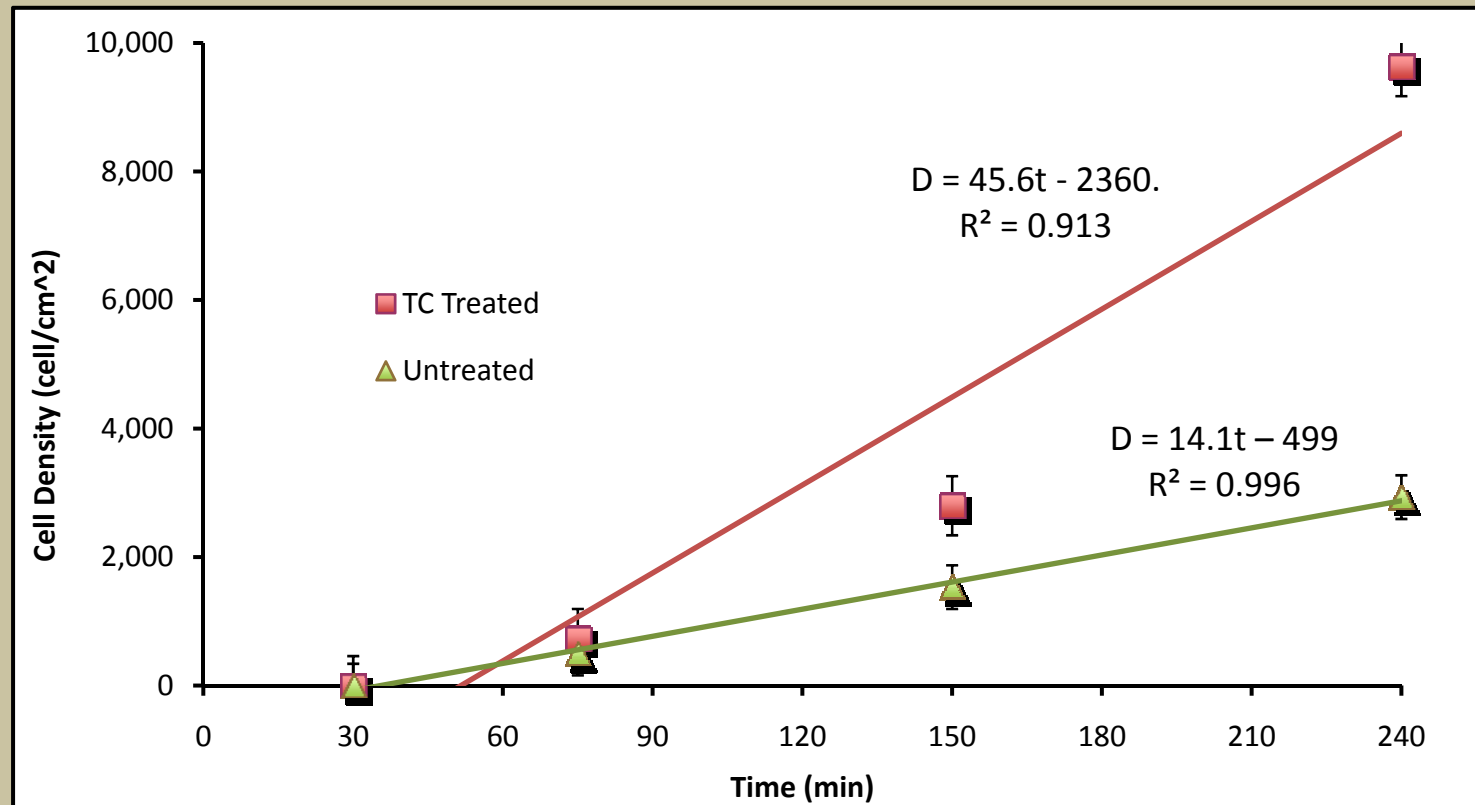
Synthesis

	Cell Proliferation Assay	Anti-PCNA Staining Assay
Conditions	Media with 1, 5, and 10% FBS	
Variable	Cell proliferation over time	Number of cells in S-Phase
Time Points	4, 24, 72, and 144 hours	48 hours only
Result	As % FBS increases, proliferation rate increases	As % FBS increases, percentage of cells in S-Phase increases
Limitations	Cannot observe individual cells dividing, so no evidence of cause of proliferation rate change	Measures rate at a single time-point, so no evidence linking FBS content to proliferation rate
Benefits of Other Assay	Proliferation rate changes because cells divide more or less frequently, not because divisions occur at a varying rate	When more cells are in the S-phase, the proliferation rate is greater, so more cells in the S-phase relates directly to more cell divisions

Cell Attachment Assay Methods

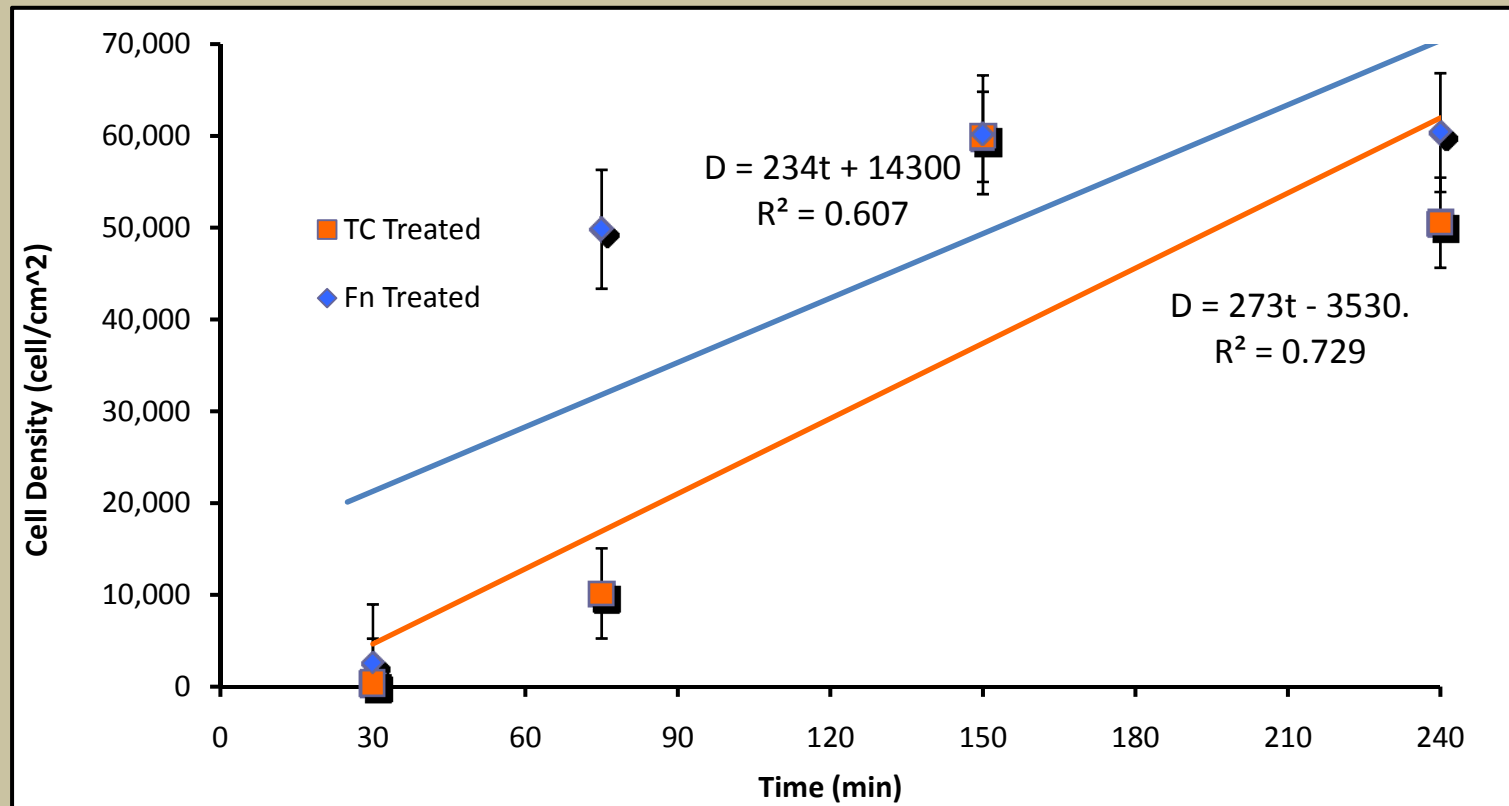
- Dilute cells to 10,000 cells/mL
- Seed 12 wells in each of untreated, TC treated, and Fibronectin (Fn) treated 24 well plates
- At 30, 75, 150, and 240 minutes, measure density of attached cells in 3 wells of each condition using CC
- Since using partner data, include TC treated plate data in both cases for comparison

Cell Attachment is Faster with TC Treated Plate than Untreated Plate



Cell attachment occurs at a higher rate on a TC coated surface than on an untreated surface. A paired t-test gives $P=0.001$ for cell density measurements at 240 min. (Data from XXX)

Cell Attachment is Similar with Fn Treated and TC Treated Plates



Cell attachment seems to occur at a higher rate on a Fn coated surface than on an untreated surface. A paired t-test gives $P=0.125$. Thus, the difference is not statistically significant.

Summary

- HDF Cell proliferation rate has a positive correlation with increasing percent FBS to 10%
 - Shown in Cell Proliferation Assay
 - Explained with Anti-PCNA Staining
- An Increasing number of HDF cells are in S-phase at any given time as percent FBS increases to 10%
 - Shown in Anti-PCNA Staining
 - Connected to proliferation rate with Cell Proliferation Assay
- HDF Cell attachment occurs more rapidly on TC and Fn treated surfaces than on untreated surfaces
 - Shown in Cell Attachment Assay