

Effects of Serum Concentration and Well Type on HDF Behavior In Vitro

2/13/08

Basis of Investigation

- To analyze the effects of serum concentration in cell media on HDF cell proliferation
 - Anti-PCNA Assay
 - Proliferation Assay
- To determine the effects of different polystyrene wells on the rate and degree of HDF cell attachment
 - Quantitative Cell Attachment Assay

Measuring HDF Attachment: Quantitative Cell Attachment Assay

- Seeded 10,000 cells in TC-treated, untreated, and Fn-coated polystyrene wells
- Incubated wells of each polystyrene subtype for four different time periods (30min, 1hr 15min, 2hr 30min, 4hr)
- Removed unattached cells with three PBS washes
- Counted attached cells with light microscope

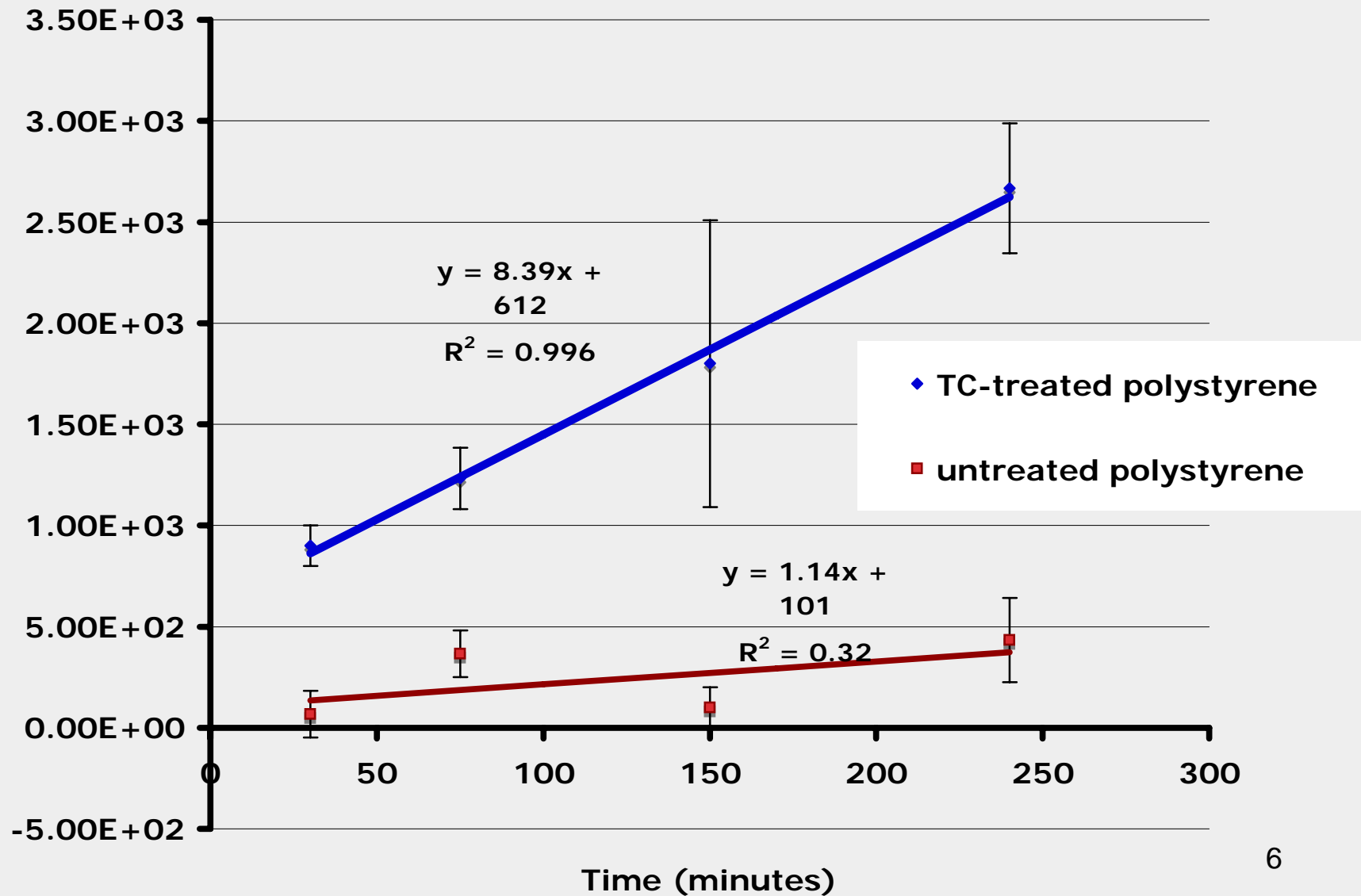
Measuring Cell Proliferation: Anti-PCNA Assay

- Seeded 20,000 cells into wells containing DMEM with 1, 5, and 10% serum
- Incubated cells for 2 days
- Used anti-PCNA staining and a light microscope to estimate fraction of dividing nuclei
- The nuclei of cells committed to division appeared red
- Non-dividing cell nuclei appeared blue

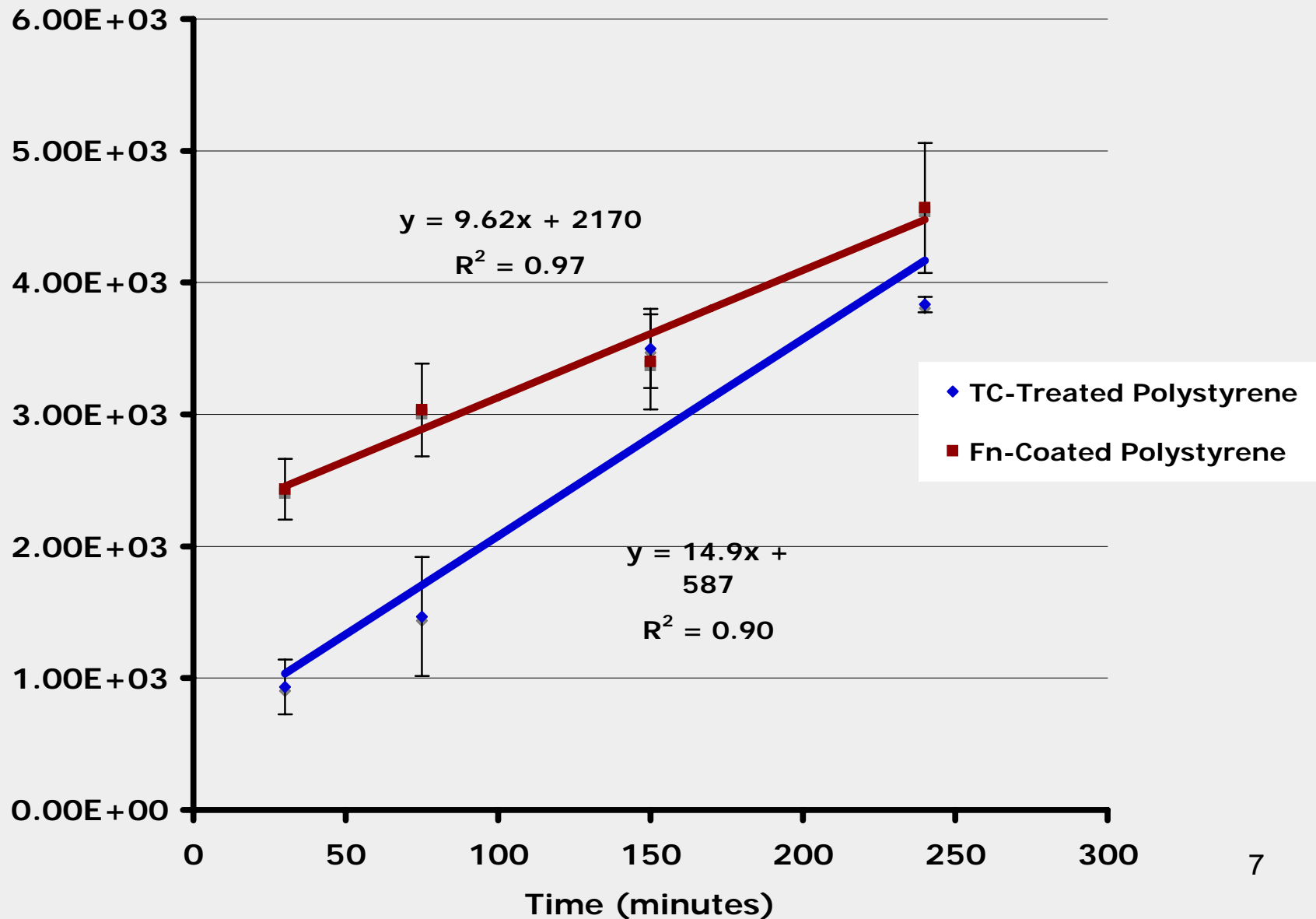
Measuring Cell Proliferation: Cell Proliferation Assay

- Plated 5,000 cells into wells containing DMEM with 1, 5, and 10% serum
- Cells under each condition were incubated for different time periods (4hr, 2 days, 5 days, and 7 days)
- Counted cells using a coulter counter

TC-Treated Wells Yield Larger Rate of HDF Attachment and Higher Cell Density



Fn-coated Wells Yield Higher Cell Density and Lower Attachment Rate



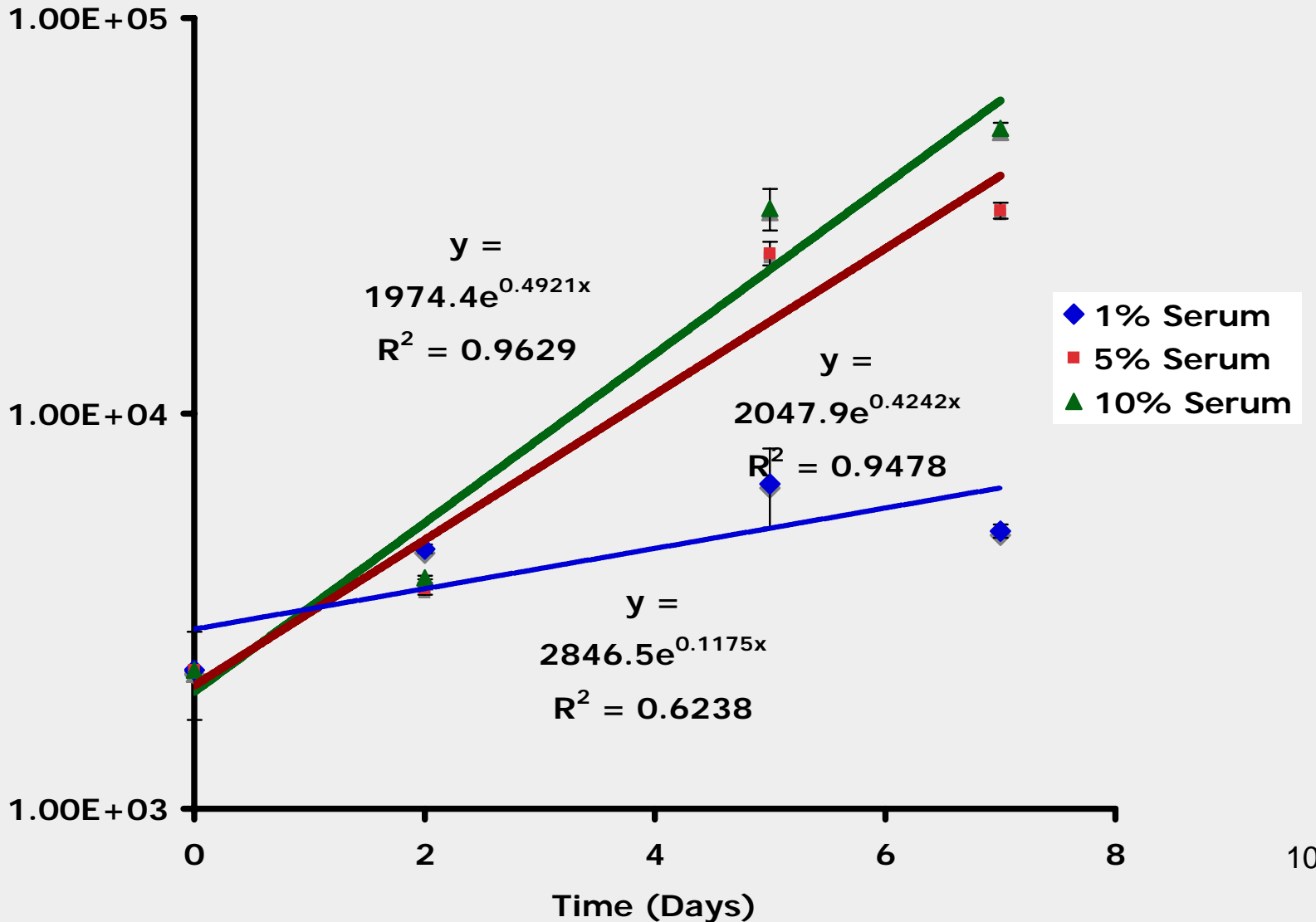
Effects of Well Composition on HDF Attachment

- TC-treated and Fn-coated polystyrene plates facilitate HDF cell attachment
- Different well types result in variable rates of cell attachment over four hours
 - Rate of HDF attachment higher in TC-treated polystyrene than in Fn-coated polystyrene
- Different well conditions result in different numbers of attached cells after four hours
 - Number of attached cells highest in Fn-coated polystyrene ($p=.025$)

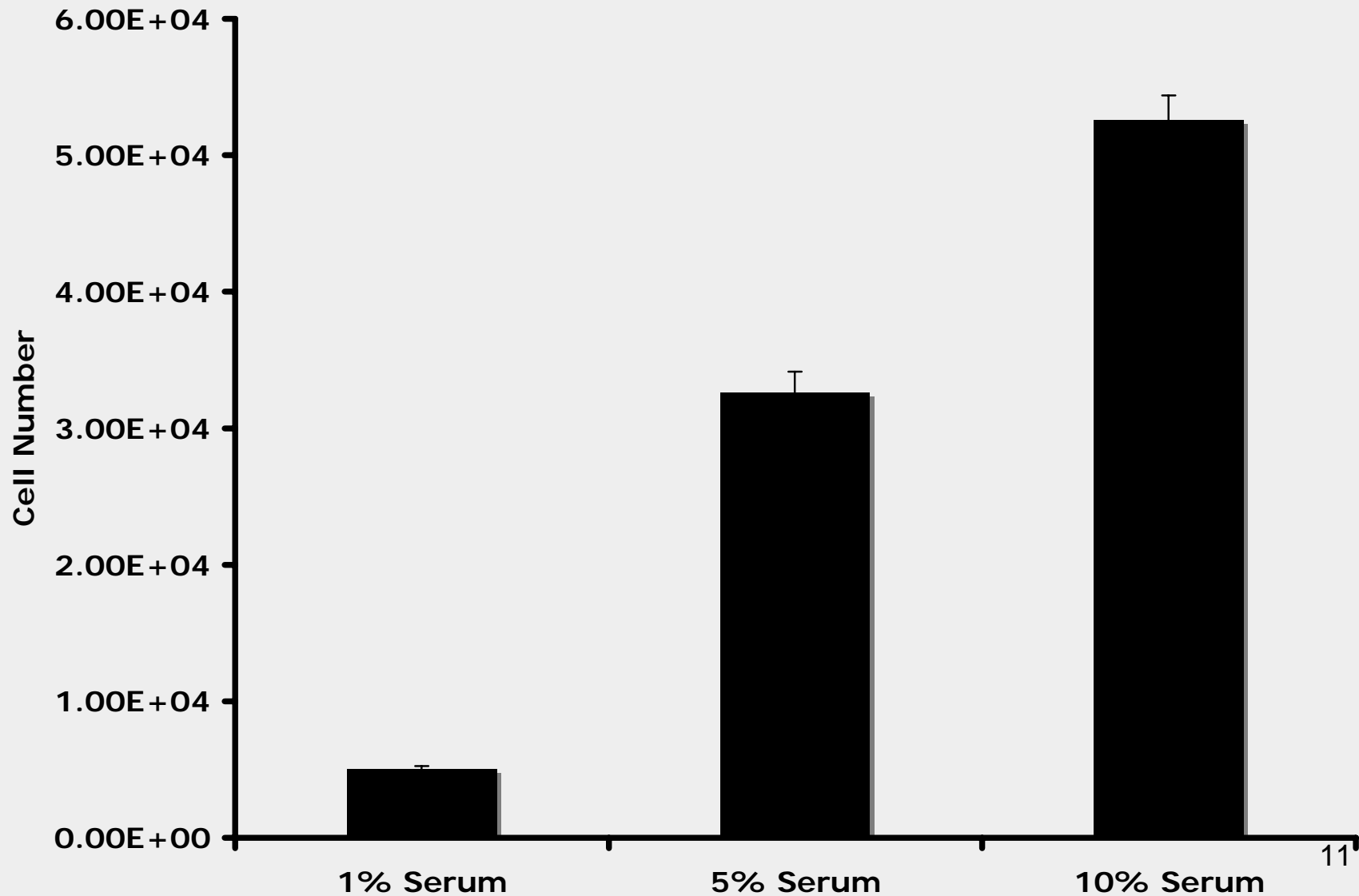
HDF Cells Incubated in 10% Serum Displayed Highest Fraction of Dividing Cells

Serum Concentration (%)	Dividing cells (%)	Non-dividing cells (%)
1	40	60
5	50	50
10	70	30

HDF Cells Incubated in 10% Serum Exhibit Fastest Proliferation Rate



10% Serum Results in Largest Cell Number After 7 Day Incubation



Effect of Serum Concentration on HDF Cell Population Growth

- Serum concentration affects cell number after seven days
 - HDF cells incubated in 10% serum had largest cell number ($p < .01$)
- Serum concentration affects HDF doubling time
 - HDF cells incubated in 10% serum displayed largest population growth rate

Results of Anti-PCNA Assay Support Proliferation Assay

- Anti-PCNA assay measured higher fraction of dividing cells in 10% serum
- Proliferation assay measured larger population growth rate in 10% serum
- Results from different assays agree
 - Rate of exponential growth of a cell population is a function of the number of cells dividing in the population
 - Larger fraction of dividing cells should correspond to faster rate of exponential growth

Serum Concentration and Well Type Affect HDF Behavior In Vitro

- Serum concentration affects total cell number and population growth rate of HDF cells in vitro
 - 10% serum yielded higher growth rates and cell numbers than 1% serum
- Polystyrene well type affects HDF attachment rate and magnitude
 - Fn-coated wells yielded higher cell density and lower attachment rates than TC-treated polystyrene
- Results indicate that in vitro conditions must be optimized for cell type of interest