



Increased Fibroblast Function With Serum and Fibronectin

YYY



Analyzing Cell Attachment and Proliferation

- To quantitatively assess the attachment of Human Dermal Fibroblast (HDF) cells to Fibronectin(Fn)-Coated, Tissue Culture(TC)-Treated, and untreated surfaces
- To determine a relationship between cell proliferation and DMEM Fetal Bovine Serum(FBS) concentration

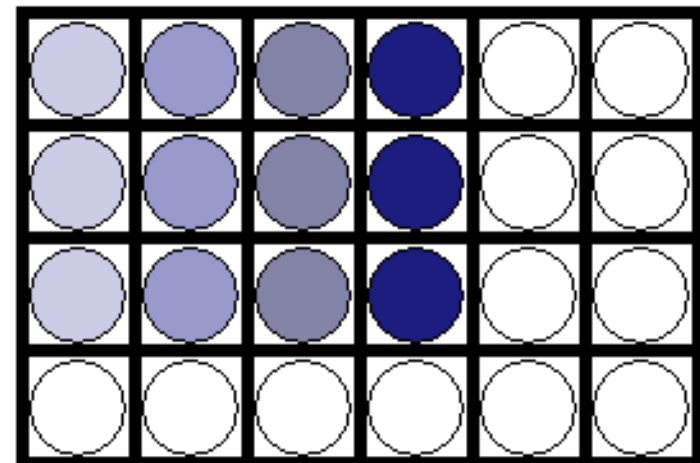
Measuring Cell Attachment

Plate Preparation

- HDF cells (P6) in a stock of 50,000 cells/mL were diluted with DMEM with 10% FBS and 1% Antibiotics to 10,000 cells/mL
- Three 24 well plates with different surface properties were prepared
 - Fn-coated
 - TC-treated
 - Untreated
- Each reading in triplicate

Time At Reading (after seeding)

:30 1:15 2:30 4:00



Sample Preparation of One Well Plate

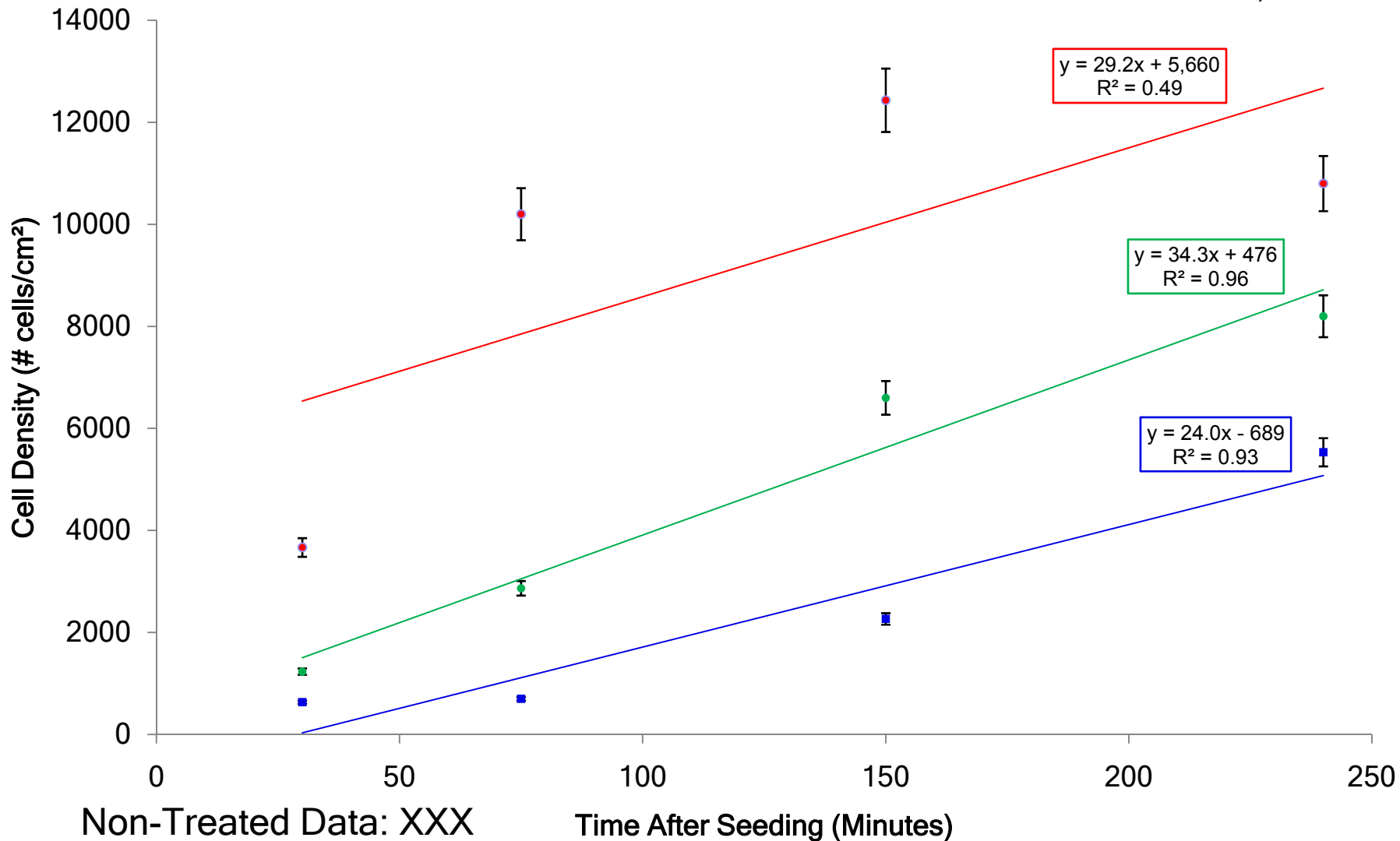
Measuring Cell Attachment

Cell Concentration Determination

- 1 mL of HDF(P6) cell dilution was added to the wells on each plate and the plates were incubated at 37°C for 4 hours
- Cell counts of wells at each time point were recorded with a Coulter Counter
- Variability in cell adhesion on the three plates was observed with a light microscope

Cell Density Increase Under Different Well Conditions

- Fn-coated Mean Values
 - TC-treated Mean Values
 - Non-treated Mean Values
 - Linear (Fn-coated Mean Values)
 - Linear (TC-treated Mean Values)
 - Linear (Non-treated Mean Values)
- P<0.001 Cell Density at 240 Minutes VS at 75 and 30 minutes
Mean+/-s.d.; n = 3



Cell Attachment Increased on Fibronectin Treated Surfaces

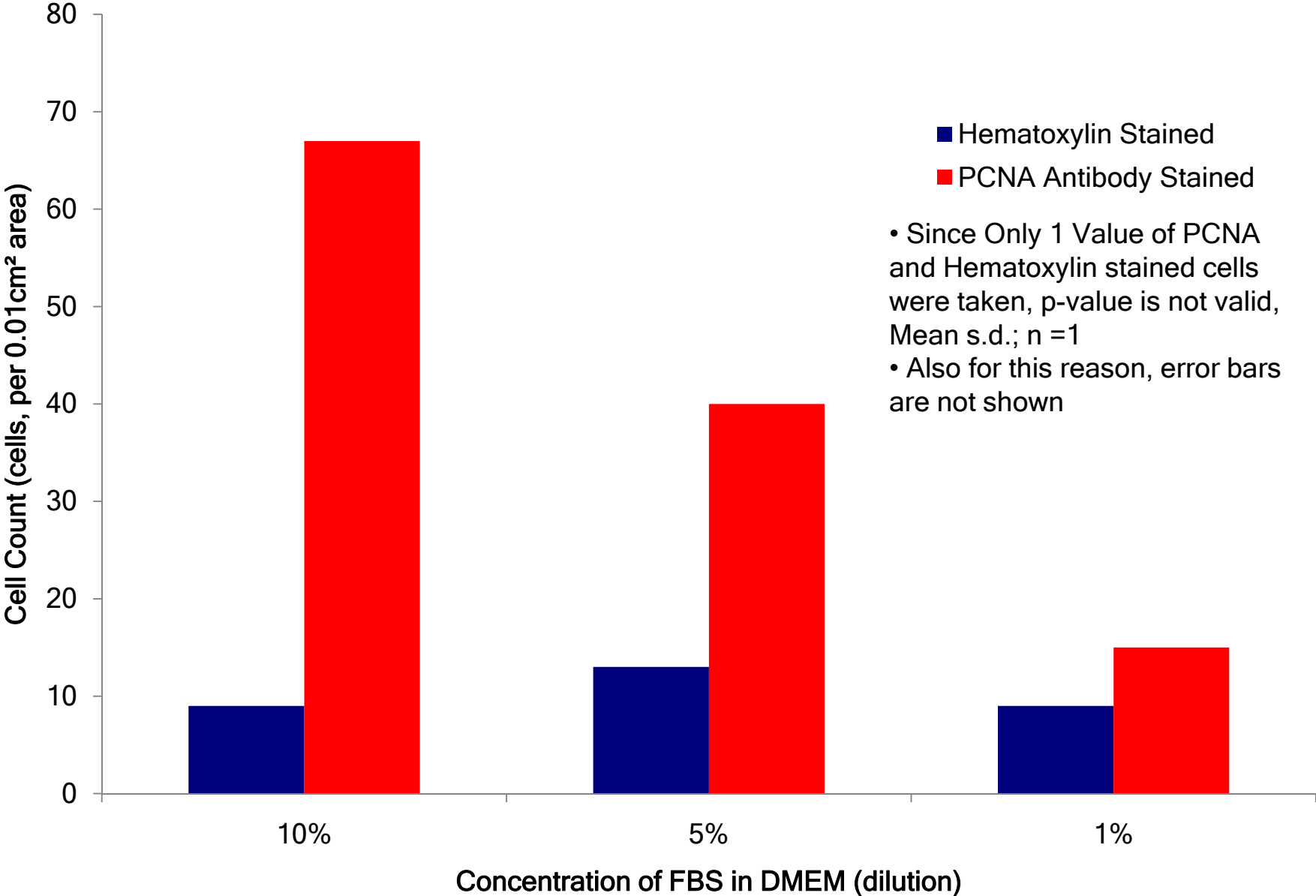
- Initially:
 - Fn induces quickest rate of cell attachment
 - Cells attach to TC-treated surfaces at a faster rate than nontreated surfaces, but slower than Fn-coated surfaces
- Largest value of cell density at all time points on Fn-coated plates
- Rate of cell attachment on TC-treated and nontreated surfaces becomes linear over time while it fluctuates on Fn-coated surfaces

Measuring Cell Proliferation

With PCNA Staining

- HDF(P6) Cells were diluted to a seeding concentration of 20,000 cells/mL in DMEM with different concentrations of FBS (10%, 5%, 1%)
- 1 mL of 20,000 cells/mL cell dilution was seeded into each well, with a total of 6 wells
 - 4 with cells diluted with 10% FBS DMEM
 - 1 with 5% FBS DMEM
 - 1 with 1% FBS DMEM
- Standard PCNA Assay procedures were followed when staining the cells
- Quantity of cells stained with antibodies and hematoxylin were observed with a light microscope without a filter

Decreasing Serum Concentration Decreases Cells in Synthesis Phase





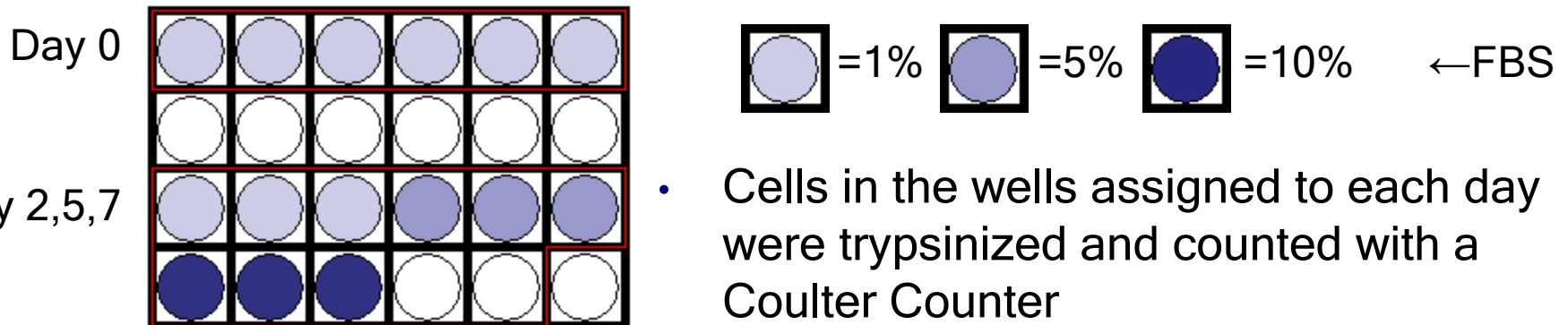
Increasing Serum Concentration Revealed More Cell Proliferation

- Cells diluted with DMEM with 10% FBS had the highest count of cells in S phase (synthesis of DNA)
- Cell count in S phase decreases with decreasing FBS concentration in DMEM

Measuring Cell Proliferation

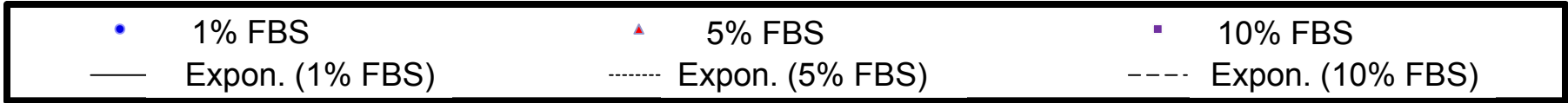
With Cell Proliferation Assay

- 1 mL HDF(P6) cell dilution (1% FBS DMEM) of 5,000 cells/mL was added to each well with a total of 33 wells and incubated for 4 hours at 37°C
- DMEM with differing concentrations of FBS was added to the wells in the following fashion:

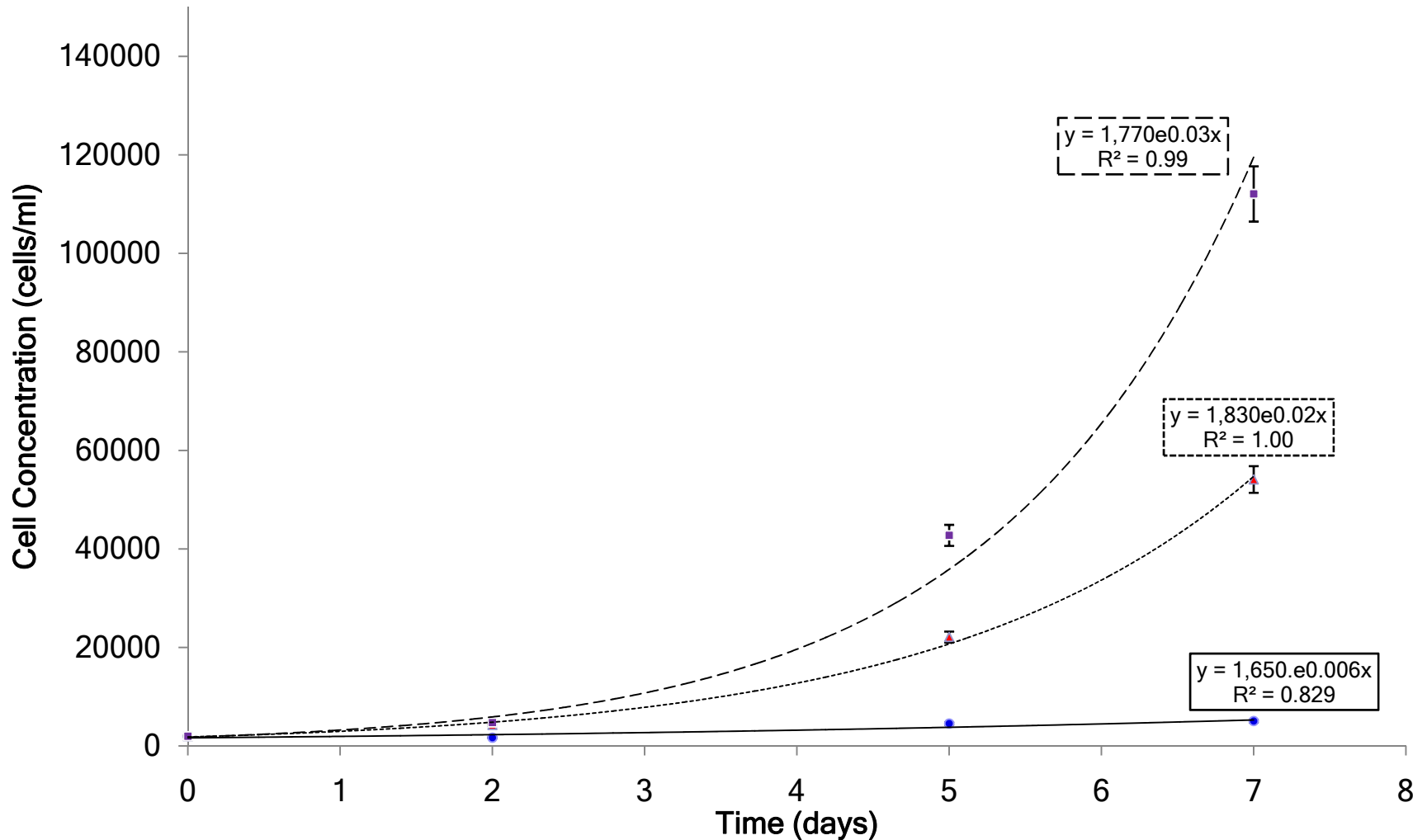


- DMEM for the remaining days was re-added on each day of measurement

Increase In Cell Proliferation With Increasing Serum Concentration Over Time



P<0.001: Cell Number from Day 7 VS from Day 2 and 5, Mean s.d.; n=3





Fibroblast Proliferation Increased with Increased Concentration of FBS

- Cells in 10% FBS in DMEM experienced the greatest exponential increase in cell concentration through a span of 7 days
- Cell concentration in 5% FBS DMEM increased exponentially over time at a slower rate than in 10% FBS
- In 1% FBS, cells didn't experience an exponential increase in cell concentration

Cell Proliferation Shown in Results of Both PCNA Assay and Cell Proliferation Assay

■ Increases in:

- cell count in the Cell Proliferation Assay
- cells that are in S phase in the PCNA Assay

→ as a result of increasing FBS concentration indicate a positive relationship between FBS concentration and cell proliferation

Fibroblast Attachment and Proliferation Increased

- Greater HDF attachment on Fn-coated surfaces than on TC-treated or nontreated surfaces
- Higher FBS concentration in DMEM leads to increased cell proliferation
- A study of cell count on Fn-coated, TC-treated, and nontreated surfaces in DMEM with 10% FBS may lead to a relationship between attachment and proliferation