Conditions for increased rate of cell proliferation in Human Dermal Cells (HDF)

YYY

Lab Partner: XXX

Objective

- To determine the ideal conditions for increasing the proliferation rate of HDF cells by varying the following test conditions:
  - Serum Concentration in media
    - 1, 5, and 10% serum with 1% antibiotic
  - Substrate Coating
    - TC-treated, Fibronectin (Fn) – coated, non-TC-treated

Cell Proliferation Assay Materials and Methods

- Proliferation was measured by seeding cells with media at:
  - 1, 5, and 10% serum with 1% antibiotic
- Cell number was calculated with a Coulter Counter at four different time points:
  - Days 0, 2, 5, and 7

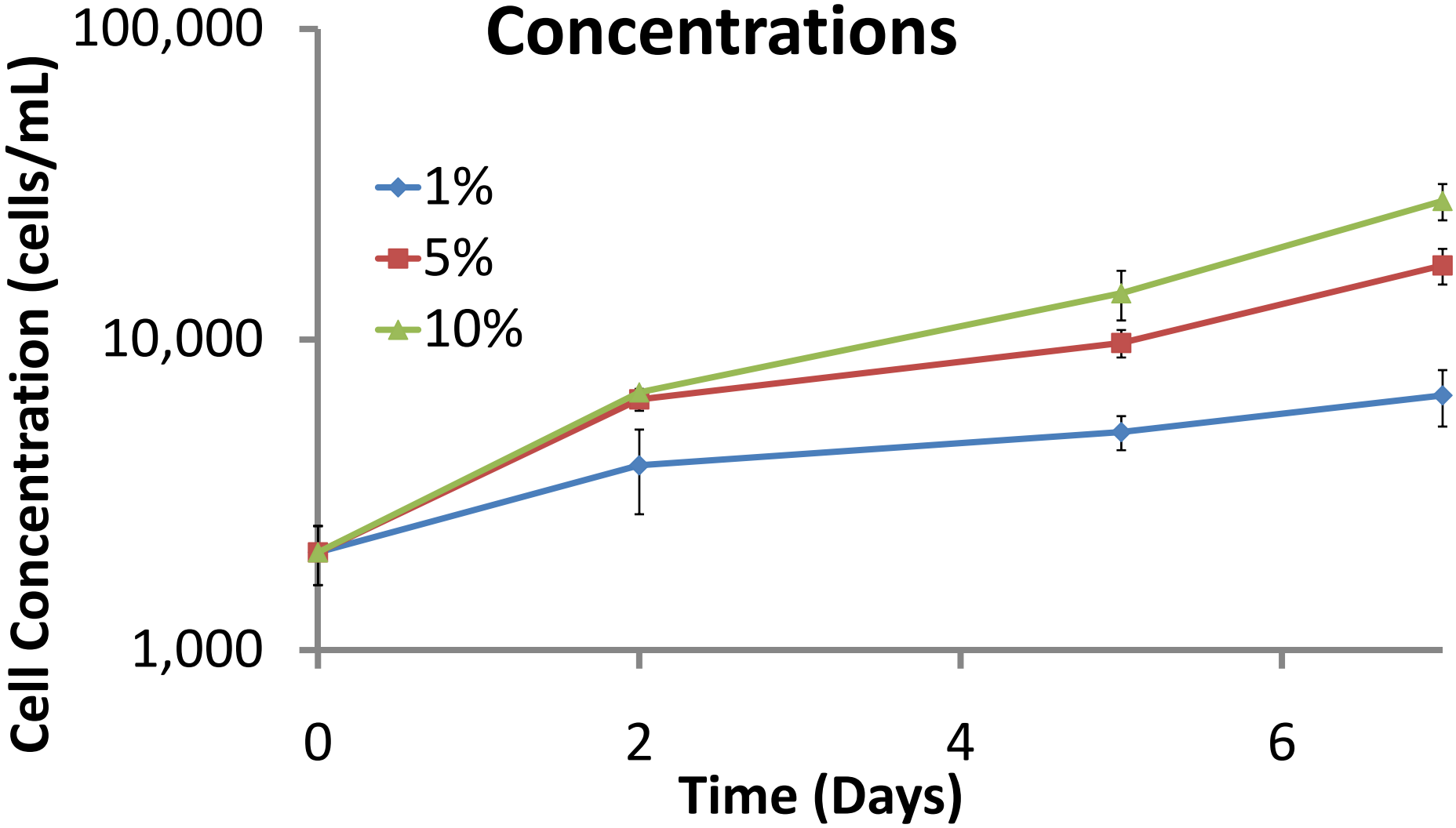
Anti-PCNA Staining Assay Methods and Materials

- Cells were grown at 1, 5, and 10% serum for two days, then stained with Proliferating Cell Nuclear Antigen (Anti-PCNA)- Mouse IgG, Anti-mouse IgG-Horse Radish Peroxidase (HRP), and hemotoxylin
- Cells were examined via light microscopy in order to in order to determine the number of cells in the S-phase. Cells in S-phase should stain red while the hematoxylin stains cell nuclei blue

Cell Attachment
Materials and Method

- Cells were seeded at 10,000 cells/well on three different test plates:
  - Fibronectin (Fn)-coated, TC-treated, and non-TC-treated
- Cells attachment was determined at four time points after seeding:
  - 30 min, 1 hr 15 min, 2 hr 30 min, and 4 hr
- Each well was thoroughly washed and cell density was determined by direct counting of cells via light microscopy

Cell Proliferation at Various Serum Concentrations



Exponential Fit	1% Serum	5% Serum	10% Serum
Equation	$y = 2063.3e^{0.1781x}$	$y = 2063.3e^{0.3194x}$	$y = 2063.3e^{0.3873x}$
R^2 Value	0.88	0.89	0.95

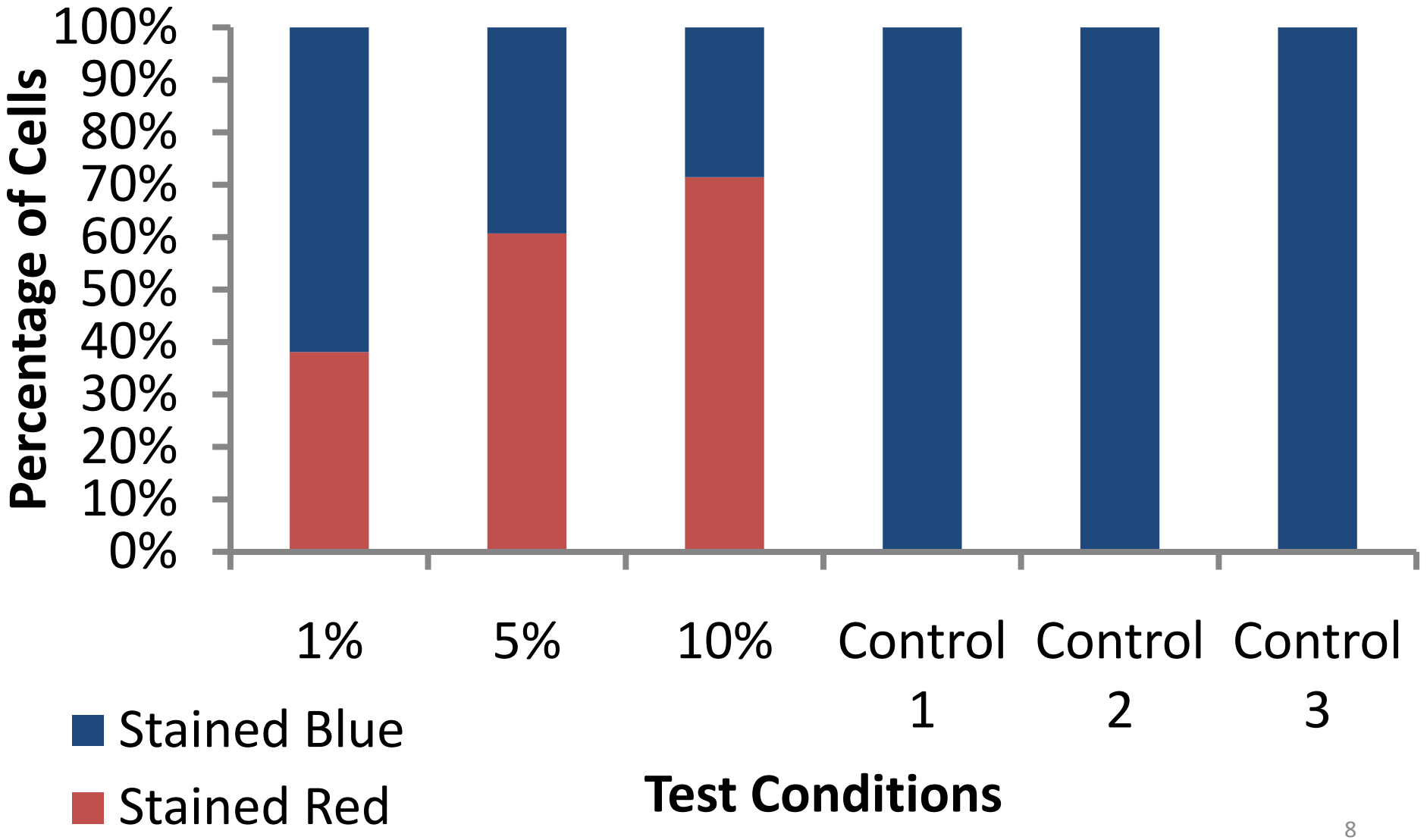
Higher serum concentrations result in higher rates of cell proliferation

P-Values between [Serum] on Day 7*	1% Serum	5 % Serum	10% Serum
1% Serum	---	$P < 0.025$	$P < 0.01$
5% Serum	---	---	$P < 0.025$

* P-Values obtained from pair-wise t-Tests between data sets

- Statistical tests show that the data sets of 1, 5, and 10% serum were statistically different
- Higher serum concentrations gave significantly higher concentrations of cells at each time point

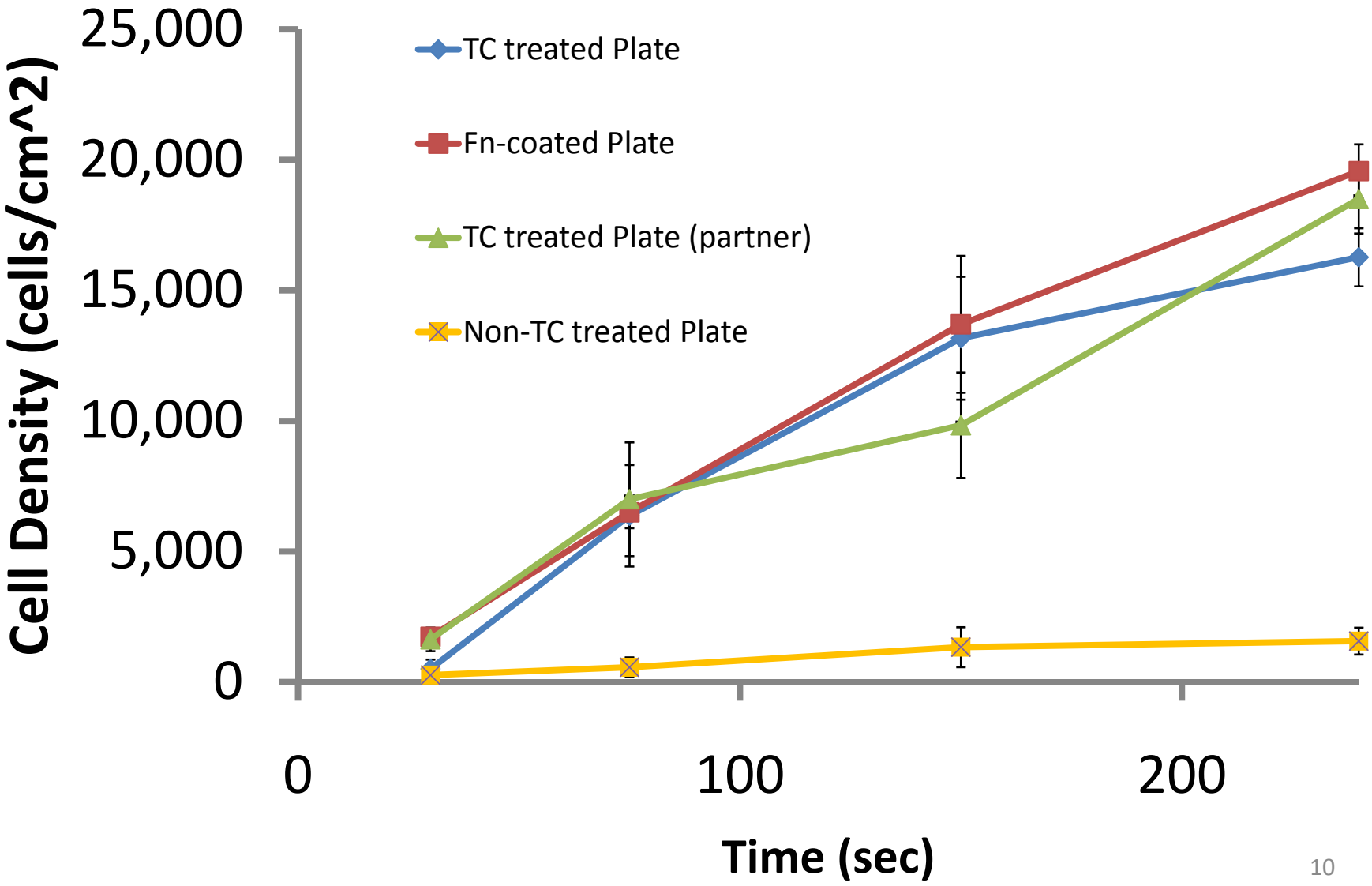
Percentage of Cells Stained by Anti-PCNA



Higher serum concentrations result in higher percentages of cells in S-phase

- Anti-PCNA staining showed that higher concentrations of serum lead to a higher proportion of cells stained red
- Red-stained cells are cells expressing PCNA and provide a measure of the percentage of proliferating cells

Cell Density from Cell Attachment Assay



Fibronectin provides the best substrate for cell attachment

P-values from comparison at 4 hrs*	TC-treated plate	Non-TC-treated plate
Fn-treated plate	P < 0.015	---
Partner's TC-treated plate	---	P < 0.002

* P-Values obtained from pair-wise t-Tests between data sets

- Analysis of the cell attachment assay shows that the number of cells attached varies according to the following relationship:

Fn-treated > TC-treated > Non-TC-treated

Higher serum concentrations lead to a higher rate of cell proliferation

- The results of the Anti-PCNA Staining Assay and the Cell Proliferation Assay show that cells grown in higher serum concentrations :
  - Have higher percentages of cells in the S-phase (Anti-PCNA)
  - Have higher populations of cells compared to lower serum concentrations at various time points (Cell Proliferation Assay)

Summary

- The Cell Proliferation and Anti-PCNA Staining Assays provide data that suggest that higher concentrations of serum in media can have a positive influence on cell proliferation rate
- The results from the Cell Attachment Assay suggest that Fn-coated surfaces provide the best surface of the three tested conditions, followed by the TC-treated plates and the non-TC-treated plates, respectively