


Effects of Serum and Surface Treatment on the Attachment and Proliferation of HDF Cells



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BIOE 342: Tissue Culture Lab

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Objectives

- ❑ To analyze the effects of serum concentration in cell media on Human Dermal Fibroblast (HDF) cell proliferation
 - Anti-Proliferative Cell Nuclear Antigen (PCNA) Assay
 - Cell Proliferation Assay
- ❑ To assess the effects of different surface treatments on the rate and extent of HDF cell attachment
 - Quantitative Cell Attachment Assay
 - Fibronectin (Fn) Attachment Assay

Measuring Cell Proliferation: Anti-PCNA Assay

- Seeded 20,000 cells/mL in wells containing:
 - DMEM with 1% fetal bovine serum (FBS)
 - DMEM with 5% FBS
 - DMEM with 10% FBS
- Incubated for 2 days
- Anti-PCNA staining
- Counted percentage of nuclei undergoing S phase with a light microscope
 - Red nuclei – cells in S phase
 - Dark blue nuclei – cells not in S phase

Measuring Cell Proliferation: Cell Proliferation Assay

- Seeded 5,000 cells/mL in wells containing:
 - DMEM with 1% FBS
 - DMEM with 5% FBS
 - DMEM with 10% FBS
- Incubated over 4 different time periods
 - 4 hrs | 2 days | 5 days | 7 days
- Counted cells using a Coulter Counter
- ANOVA pair-wise test determined if test conditions had significantly different cell numbers on Day 7

Measuring Cell Attachment: Quantitative Cell Attachment Assay

- Seeded 10,000 cells/mL on 3 different plates:
 - TC-treated
 - Untreated
 - Fn-coated
- Incubated cells over 4 different time periods
 - 30 min | 1 hr 15 min | 2 hrs 30 min | 4 hrs
- Removed unattached cells with 3 phosphate buffer saline (PBS) washes
- Counted attached cells using a light microscope

Measuring Cell Attachment: Qualitative Fibronectin Attachment Assay

- Seeded 50,000 cells/mL in untreated wells with different degrees of Fn coverage:
 - A: Control (no Fn)
 - B: Half Fn, Half Control
 - C: Fn Design
 - D: Fn-saturated
- Incubate for 2 hrs
- Observe cell adhesion and morphology in wells for Conditions A, B, C, and D using light microscope
 - Before and after PBS rinse

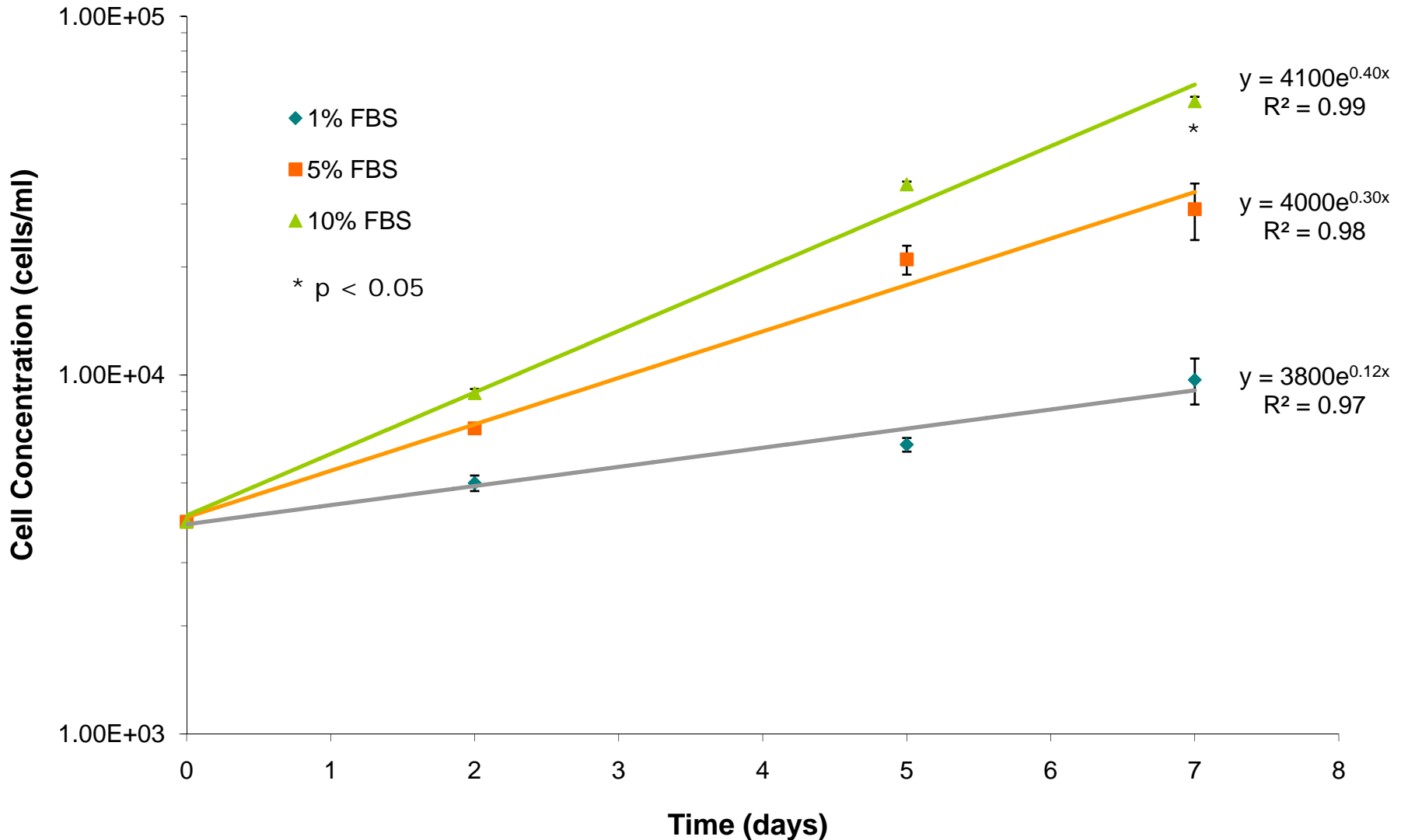
Fraction of HDF Cells in S Phase Increases with % Serum in Media

% FBS in Media	Total # of Cells	# of Red-Stained Nuclei	% of Cells in S Phase
1	70	17	24
5	110	31	28
10	130	47	36

- Increasing the amount of serum in media increases the number of cells with red nuclei (i.e. cells undergoing S phase)
- Total cell count increases with the amount of serum in media

Serum Increases the Exponential Proliferation Rate of HDF Cells

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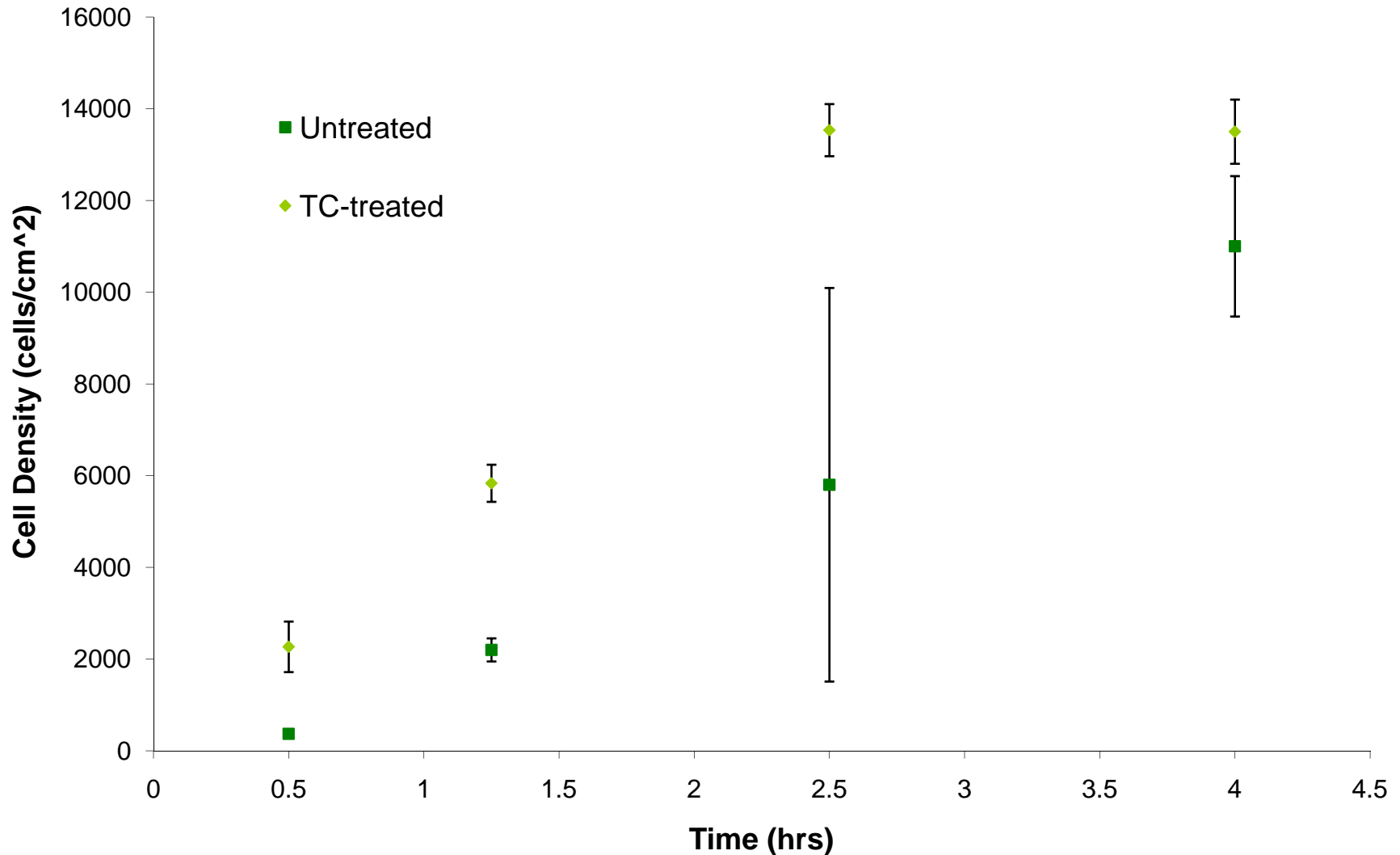
HDF Cell Proliferation Increases with Serum Concentration

- HDF doubling time shortens with increasing serum concentration
 - HDF cells incubated in DMEM with 10% FBS displayed highest population growth rate
- Total number of cells increases with serum concentration
 - Wells containing DMEM with 10% FBS had the highest number of cells on Day 7 ($p < 0.05$)

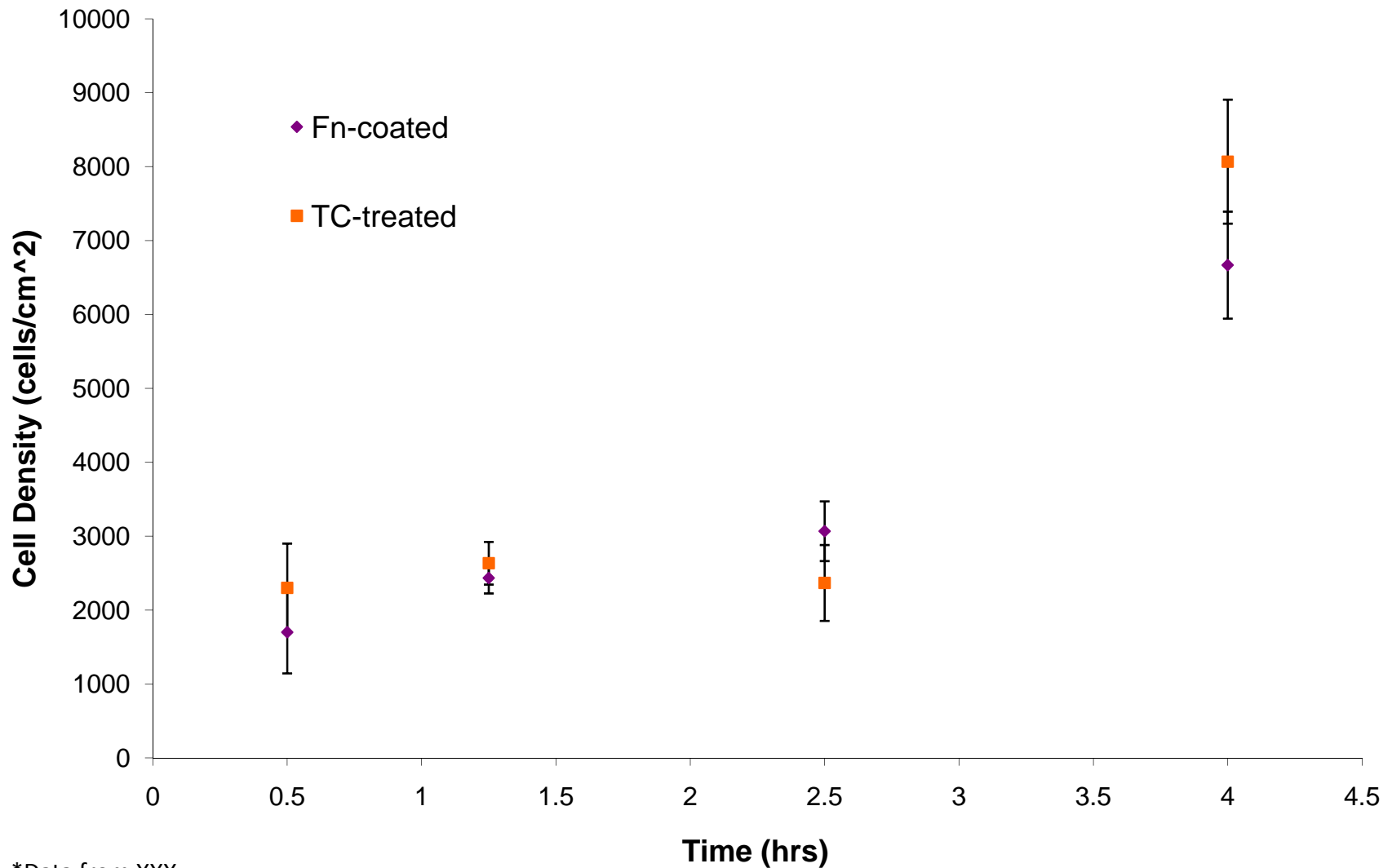
Data from Cell Proliferation Assay Supports Results from Anti-PCNA Assay

- Cells in media with 10% serum had the fastest population growth rate
- Highest percentage of cells preparing to divide (i.e. cells in S phase) observed in media with 10% serum
- Results from both assays are consistent
 - Rate of exponential growth of a cell population depends on how often cells in the population will divide
 - Larger percentage of dividing cells results in faster rate of exponential growth

HDF Cells in TC-treated Wells Have Higher Attachment Rate than Untreated Wells



HDF Cells in Fn-Coated Wells Have Lower Attachment Rate than TC-treated Wells



*Data from XXX

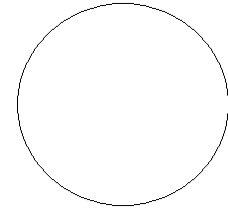
HDF Attachment Varies Among Different Surfaces

- TC-treated surfaces promote the highest rate of attachment
- Fn-coated surfaces yield a higher attachment rate than untreated surfaces
- All surfaces reach similar “final” cell densities at 4 hrs ($p > 0.05$)

Fibronectin Promotes HDF Attachment

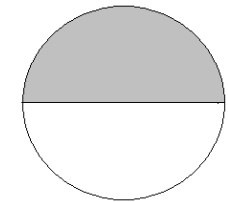
□ A: Control (No Fn)

- Very few attached cells
- Round in shape, no spreading



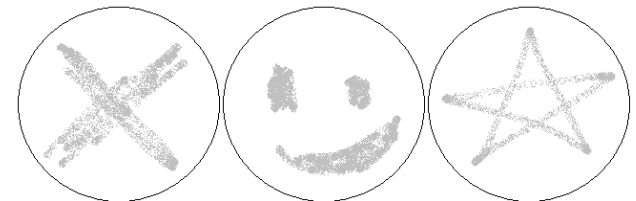
□ B: Half Fn, Half Control

- Control half: Very few attached cells
- Fn half: Elongated, attached cells



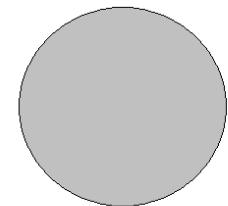
□ C: Fn Design

- Attached cells follow Fn design
- Elongated in shape, some spreading



□ D: Fn-saturated

- Entire well contains attached cells



Results from Fn Attachment Assay Correspond to Results from Quantitative Attachment Assay

- Fn enhances development of attached cell morphology
 - Fn-coated: Cells displayed spreading & pseudopodia
 - Untreated: Cells were round and did not spread
- Fn increases the number of attached cells
 - Quantitative Assay counted more cells in Fn-coated wells than in untreated wells
 - Fn Attachment Assay observed more attached cells on Fn-coated surfaces than on untreated control surfaces

Optimized Serum Concentrations and Surface Treatment for HDF Culture

- Serum concentration in media
 - 10% FBS had the most positive effect on HDF proliferation
 - 5% FBS improved cell proliferation more than 1% FBS
- Surface treatment
 - TC-treated surfaces yielded the highest attachment rate
 - Fn-coated surfaces are superior to untreated surfaces in promoting cell attachment and proliferation
 - Final attachment density unaffected by surface treatment
- Consider serum concentration and surface treatment when optimizing culture conditions for cells