

Effects of surfaces and serum concentration on HDF attachment and proliferation

YYY

February 11, 2009

BIOE 342: Laboratory in Tissue Culture

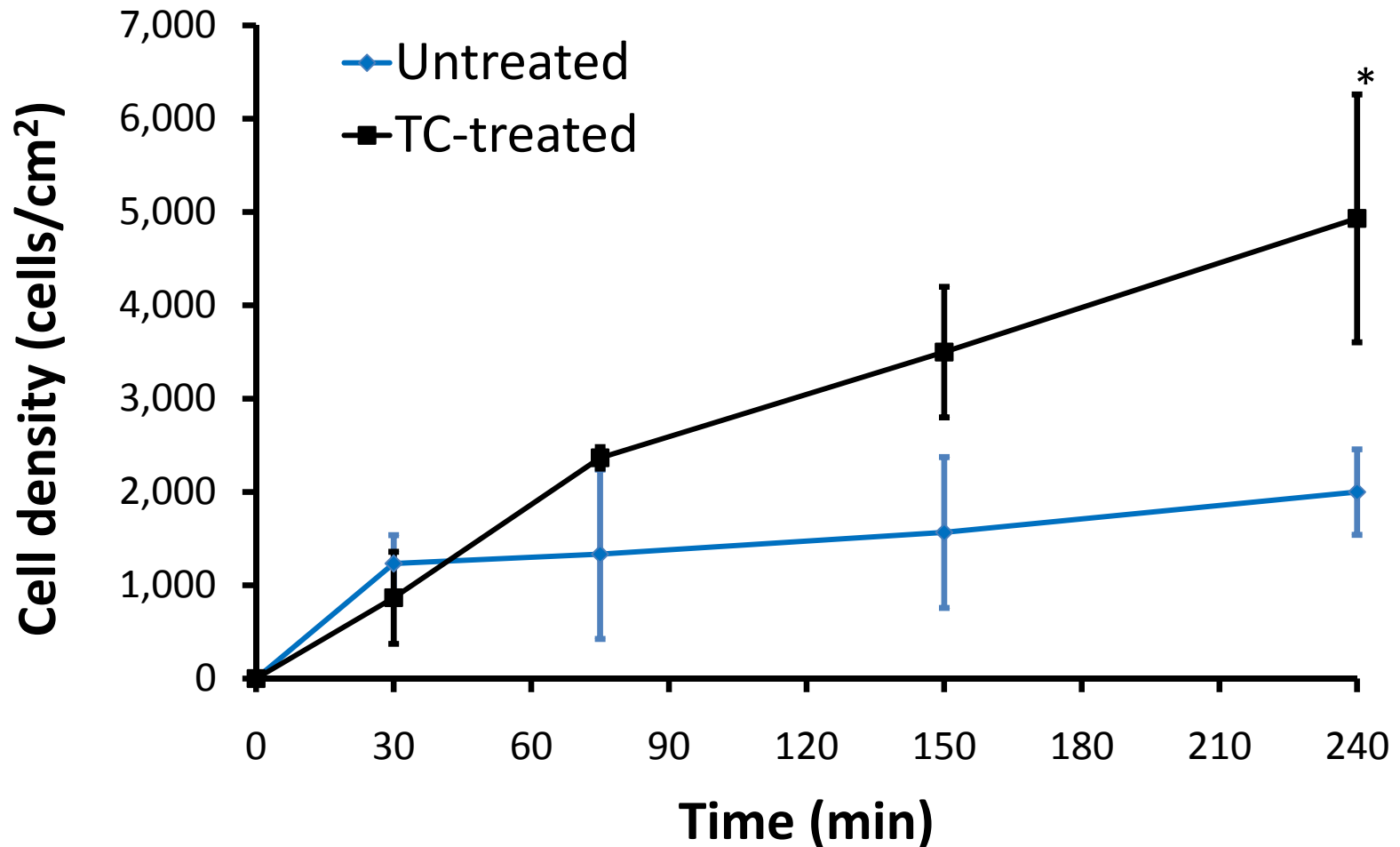
Objectives

- To determine the influence of surface modification on cell attachment
- To analyze the effects of serum concentration on cell proliferation & attachment

Cell attachment assay methods

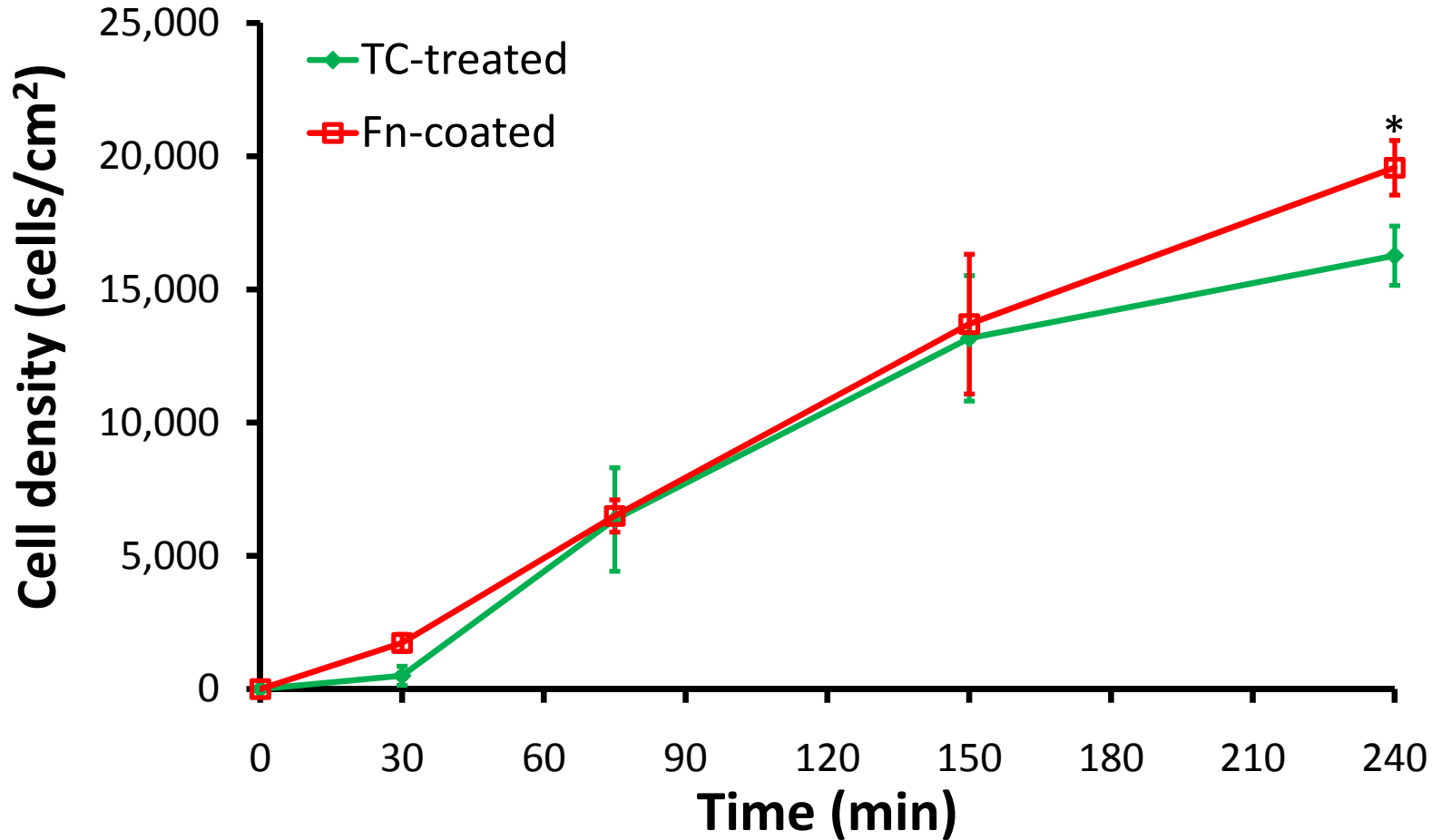
- Human dermal fibroblasts (HDF) seeded on surface-modified polystyrene plates
 - Tissue culture treated (TC-treated)
 - Fibronectin coated (Fn-coated)
 - Untreated
- Cell density was measured 30, 75, 150, and 240 minutes after seeding using a light microscope

Surface treatment induces greater HDF attachment



* p<.05 (T-test)

Fn enables more HDF attachment



Data provided by XXX

* $p < .05$ (T-test)

Immunocytochemistry methods

- Anti-Proliferating Cell Nuclear Antigen (PCNA)
- HDF cells incubated for 2 days in DMEM w/ 1% antibiotic and 1%, 5%, 10% FBS (fetal bovine serum)
- After cells fixed, PCNA localized
 - 1^o antibody (Ab): Anti-PCNA-mouse IgG
 - 2^o Ab: Anti-mouse IgG-horseradish peroxidase (HRP)
 - HRP substrate: AEC (3-Amino-9-EthylCarbazole)
- Observed nuclei staining using light microscopy
 - Red: in S phase (HRP & AEC reaction)
 - Blue: not in S phase (hematoxylin)

More cells in S phase at higher serum concentration[‡]

% FBS	% Confluency	% Red nuclei
1	20-30	75
5	15-50	75
10	25-70	95
Control*	10-85	0

- With increasing serum concentration
 - Higher confluency
 - More nuclei stained red

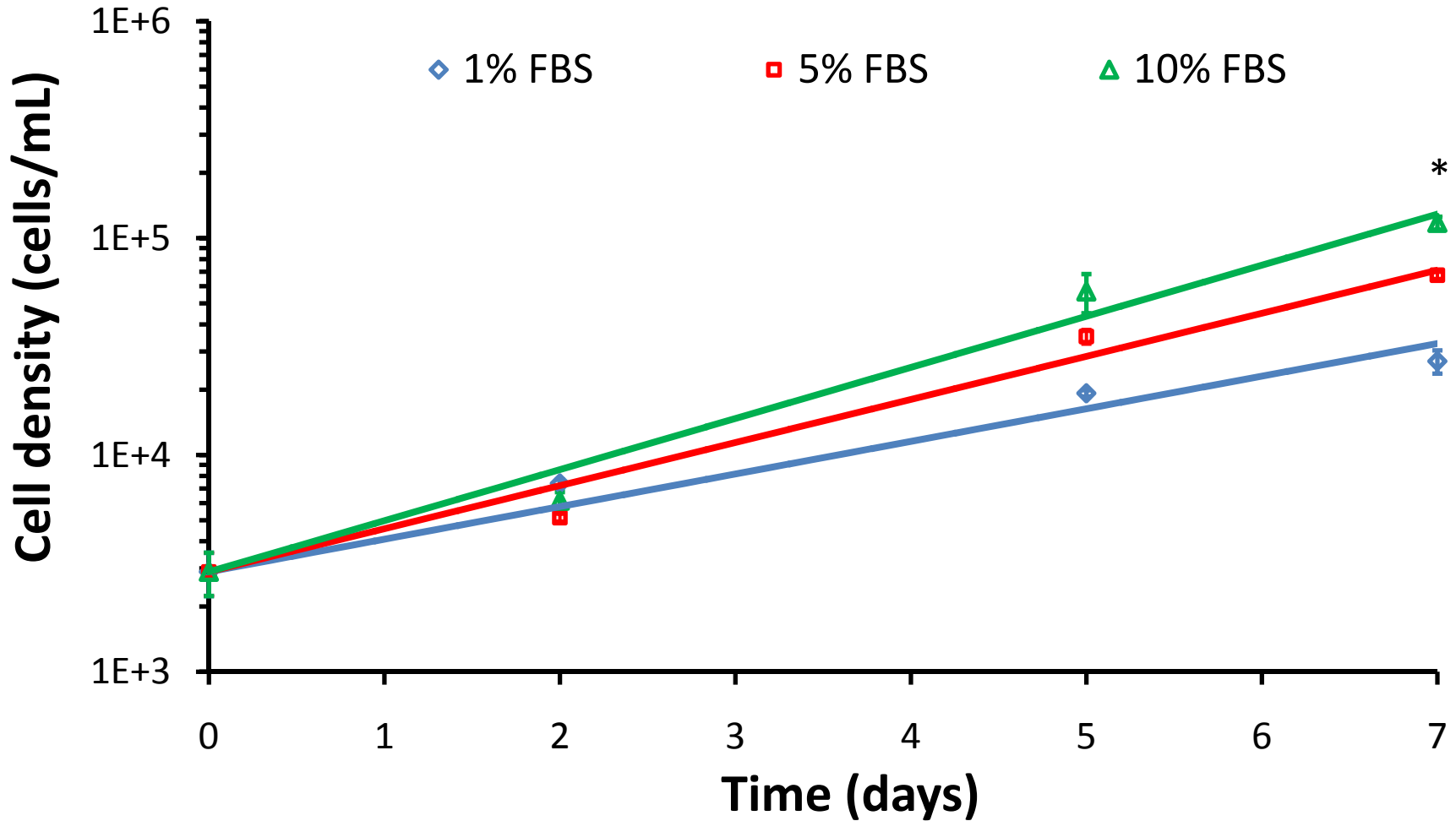
* 3 control wells

[‡] No statistical analysis available

Cell proliferation assay methods

- Cells plated in DMEM w/1 % FBS and 1% antibiotics
- Cells grown in 3 different growth conditions
 - DMEM w/1% FBS
 - DMEM w/5% FBS
 - DMEM w/10% FBS
- Cell concentration determined on days 0, 2, 5, and 7 using Coulter Counter

Cells exhibit exponential growth



* $p < .001$ (ANOVA); all are significantly different ($p < .001$; Tukey's HSD)

Increasing serum concentration decreases doubling time

Serum (% FBS)	Exponential rate (days ⁻¹); R ² *	Doubling time (days)
1%	0.35; 0.96	2.0
5%	0.46; 0.98	1.5
10%	0.54; 0.98	1.3

- Cell culture in 10% FBS had significantly higher population than those in 1% and 5% FBS

* Assuming all plates seeded at same concentration of cells

Serum provides essential signals for cell growth and division

Increase FBS concentration



Increased number of cells in S phase



Increased proliferation rate



Reach confluency faster

- Increase serum content
 - Increase growth factors
 - Increase other cellular signals
- More cells continue through the cell cycle
- Population doubles faster
- Supported by data from cell proliferation and anti-PCNA assays