



HDF Cell Proliferation and Attachment in Vitro

Objectives

- Establish a relationship between % serum and cell proliferation.
- Observe the effect of Fibronectin (Fn) on cell attachment.
- Analyze the quantitative difference between TC-treated, untreated, & Fn-coated plates on cell attachment.

Cell Proliferation Assay

- Seed HDF cells containing 5,000 cells/mL onto 2 TC-treated plates.
- 2 Test Variables:
 - Time (0, 1, 3, or 6 Days)
 - Day 0 cells were placed only in 1% FBS serum
 - % Serum(1, 5, or 10 % FBS)
- Incubate cells based on condition time.
- Use Coulter Counter to measure cell growth and replenish media for days not counted.

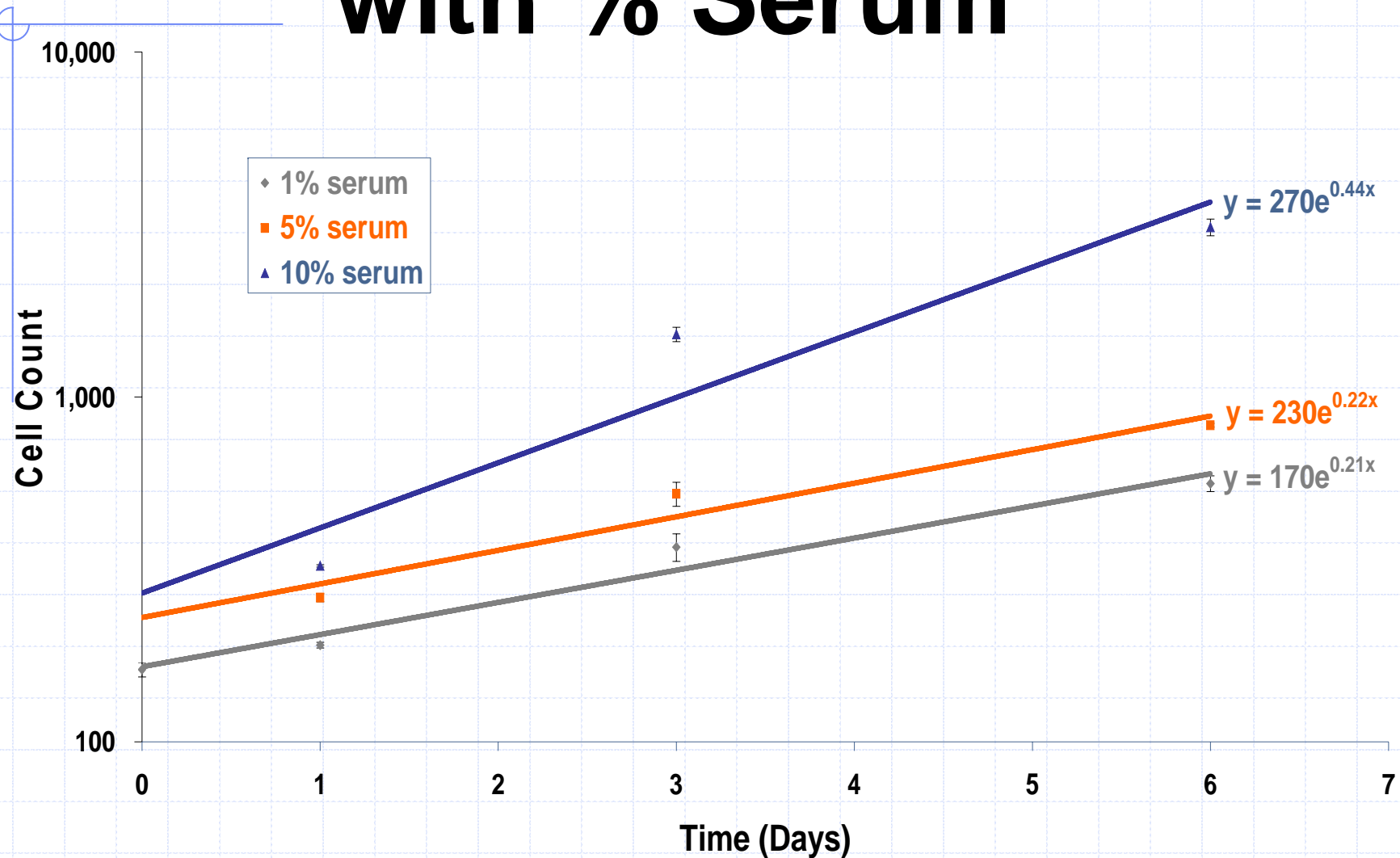
Fibronectin Attachment Assay

- Coat Fibronectin (Fn) onto untreated plate with these 4 Test Conditions:
 - 3 control wells (PBS)
 - 3 Half Fn/Half PBS
 - 3 “X” pattern Fn-coated
 - 3 Fn-coated
- Seed HDF cells containing 50,000 cells/mL to untreated plate & incubate for 2 hours.
- Observe attachment with a light microscope before and after washing with PBS.

Quantitative Attachment Assay

- 2 Test Variables:
 - Time (30, 75, 150, & 240 min)
 - Plate (TC-treated, untreated, or Fn-coated)
- Seed HDF cells with 10,000 cells/mL onto varying plates.
- Incubate cells based on condition time.
- Wash with PBS and determine attached cell density using a light microscope.

Cell Growth Increases with % Serum



Relationship between % FBS and Cell Growth

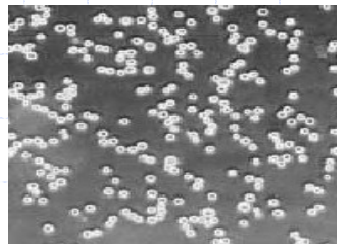
- Doubling Time = $\ln(2)/\text{slope of linear fit}$

% FBS	Doubling Time (Days)
1	3.2
5	3.1
10	1.6

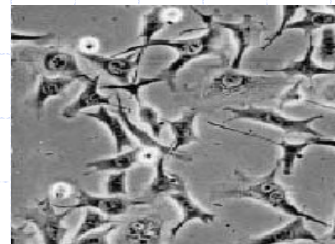
- Statistically significant differences are found within Day 6 using One Way Anova and Tukey Test ($p < 0.001$):
 - 1% vs. 10% FBS
 - 5% vs. 10% FBS

Fn Aids HDF Cell Binding

Control

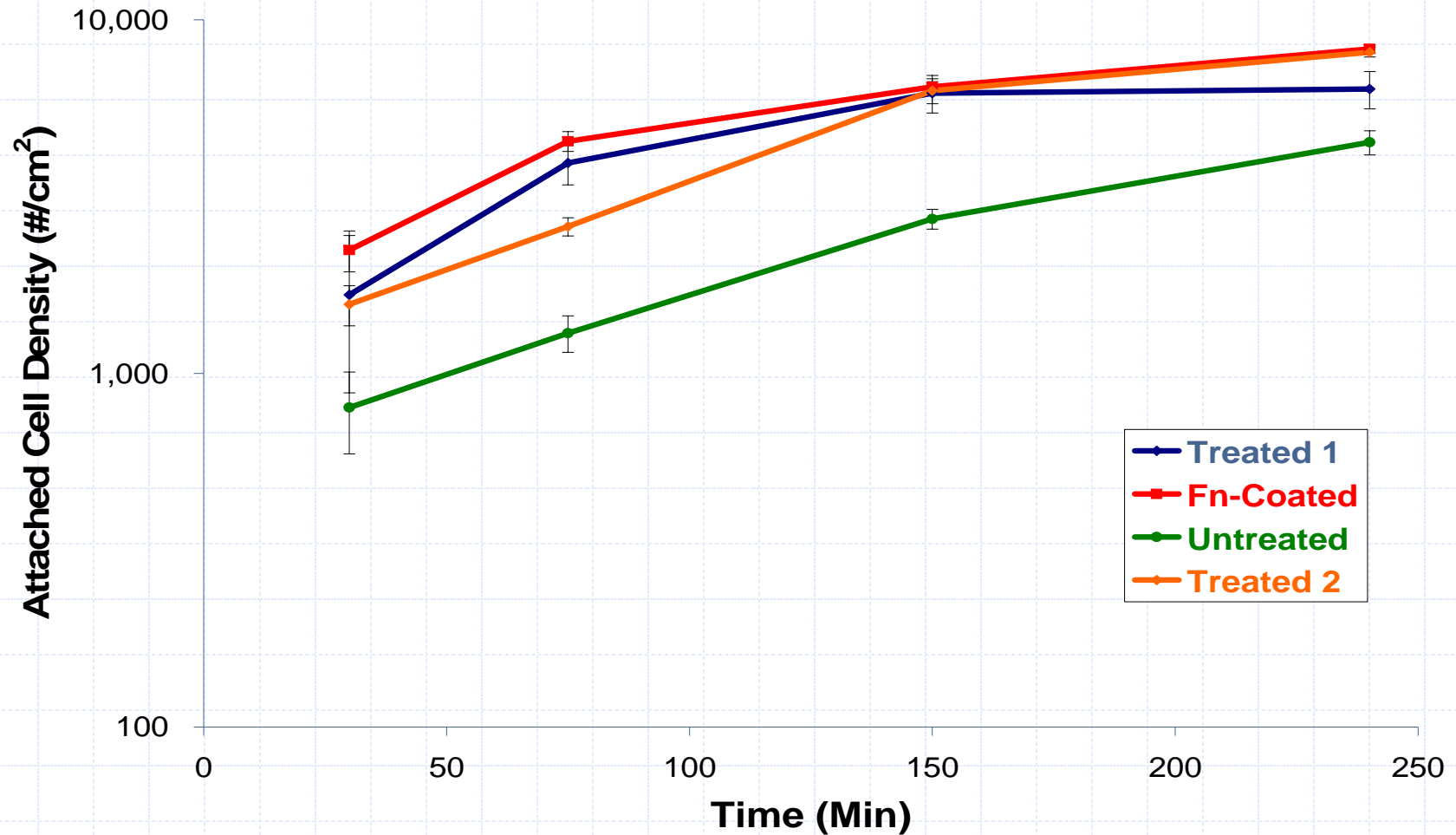


Fn-Coated



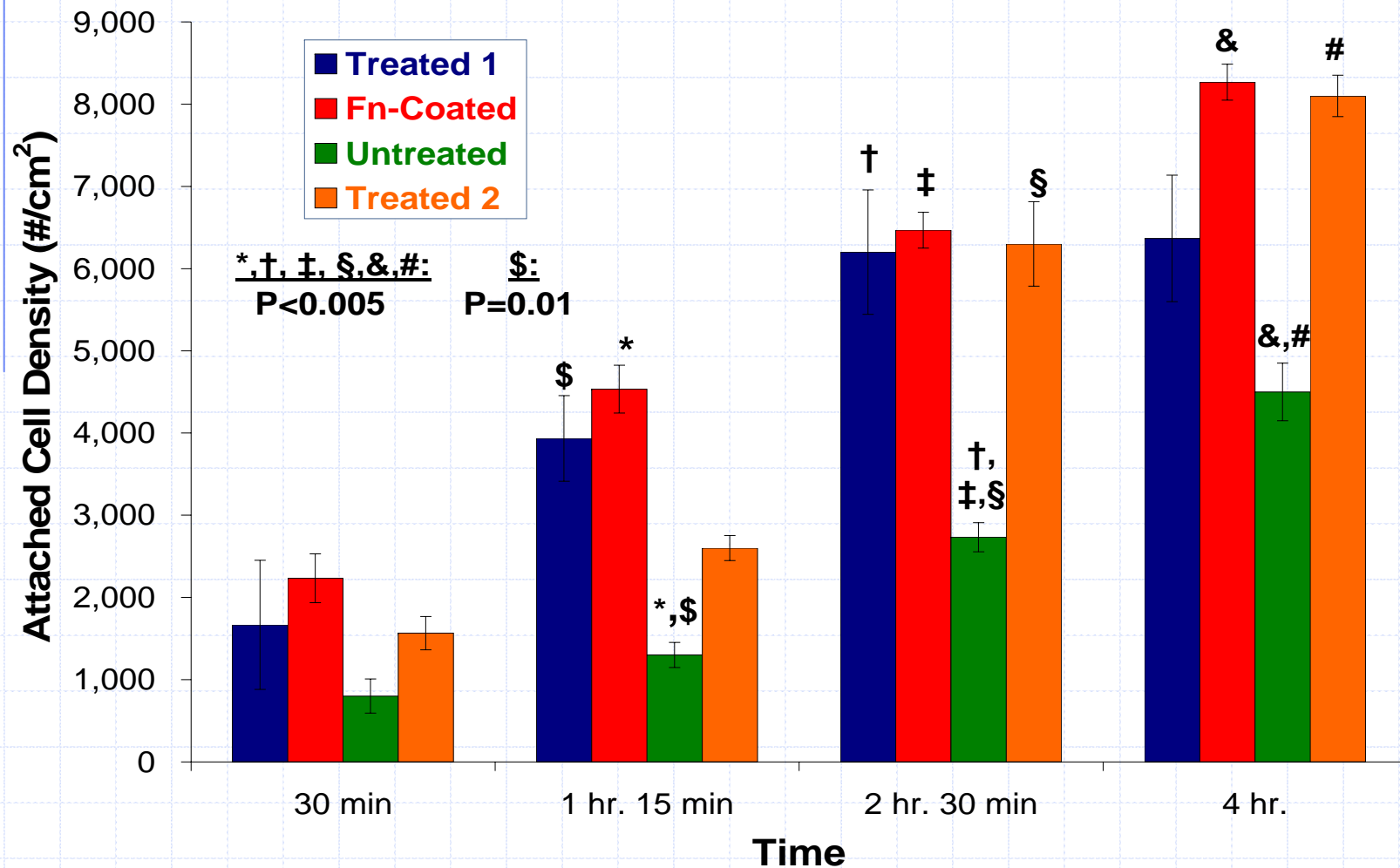
Conditions	Observations(After PBS Wash)
Control	Few attached cells
Half Fn/Half Control	Half attached, half blank design is clearly observed (elongation of attached cells)
“X” coated Fn	“X” design of attached cells is observed (not as clear as Half Fn/Half Control design)
Fn-coated	Covered in attached cells (elongation and extension of pseudopodia seen)

Fn & TC-Treatment Speed Attachment



- Highest rate of attachment from 30-75 min. Levels out at 2 hr. 30 min (150 min).
- Fastest rate of attachment is Fn.

Fn & TC-Treatment Increases Cell Attachment



Statistics were done using One Way Anova and Tukey Test for pairwise comparisons within time points.

Fibronectin vs. Quantitative Attachment

Fibronectin Attachment Assay	Disadvantages	<ul style="list-style-type: none"> •Qualitative •Rate of cell attachment can't be determined •Only tests 1 condition (Fn)
	Advantages	<ul style="list-style-type: none"> •Takes less time (not time conscious) •Small room for error.
Quantitative Attachment Assay	Disadvantages	<ul style="list-style-type: none"> •Dead cells and particles are counted in Coulter Counter •Large room for error.
	Advantages	<ul style="list-style-type: none"> •Tests multiple conditions (TC-treated, untreated, Fn-coated) •Tested at varying time pts. •Quantitative

Comparison of Results

Type of Assay	Results
Fn Attachment	<ul style="list-style-type: none">• visible differences in morphology and attachment of the Fn coated and control• easier binding of HDF cells with Fn
Quant. Attachment	significantly faster rate of attachment with Fn & TC-treated plates.

- **Fn Attachment Assay:**
 - Results are almost always accurate b/c of its qualitative nature.
 - Less information is obtained.
- **Quantitative Attachment Assay:**
 - Results have large variances that will sometimes distort the expected result.
 - More meaningful results.

Conclusions

- **Higher serum concentrations in media increase rate of cell replication.**
 - Faster doubling time calculated for 10% FBS than 5 or 1% FBS
 - Cell Proliferation observed at Day 6 gave significantly different cell counts for 10% FBS compared to 1 & 5% FBS ($p < 0.001$)
- **Fibronectin promotes attachment**
 - Easier binding of HDF cells to Fn surfaces
 - Fn changes cell morphology by promoting the elongation of cells.
- **Fn & TC-treatment increase rate of attachment**
 - Faster attachment of HDF cells to Fn-coated & TC-treated plates
 - Fn-coated & TC-treated plates have significantly larger attached cell density than untreated plates