



Attachment and Proliferation of Human Dermal Fibroblasts

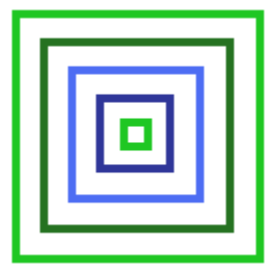
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Objectives



- To study the effect of different surface treatments on HDF attachment
 - Fibronectin Attachment Assay
- To measure the proportion of attached HDF cells over time
 - Quantitative Cell Attachment Assay
- To analyze the effects of various media on HDF cell proliferation
 - Cell Proliferation Assay



Fibronectin Attachment Assay



- 4 test conditions
 - Non-TC-treated
 - Half F_n treated, half non-TC-treated
 - Design painted in F_n
 - Completely F_n covered
- Seed 50,000 cells/well
- Allow to incubate for 2 hr
- Determine adhesion, morphology and spreading with a light microscope



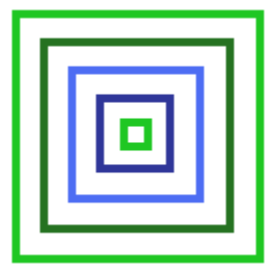
Quantitative Cell Attachment Assay



- Test 3 conditions
 - TC-treated, Untreated, F_n -coated polystyrene
- Check attachment at 4 times
 - 30 min, 1 hr 15 min, 2 hr 30 min, 4 h
- Determine cell number, morphology, shape, and spreading with light microscope





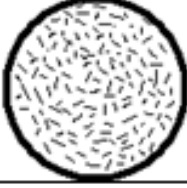
Cell Proliferation



- Test 3 media conditions
 - Media with 1%, 5%, 10% FBS
- Test 4 time points
 - 4hr, 1d, 3d, 6d
- Seed 5,000 cells/well
- Estimate cell density with light microscope
- Determine cell concentration with Coulter Counter

Cells Form Weak Attachments With and Without Fibronectin

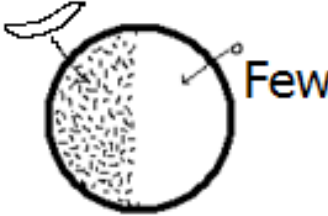


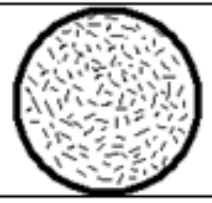
After 2 h incubation- No Wash

Condition	Cell Adhesion	Cell Morphology	Pattern	Extent of Spreading
Non-TC Treated	Few	Circular	No pattern	None
Half F_n Treated	Many	Elongated and circular		Half
Design drawn in F_n	Many	Half elongated, Half circular		On Design
F_n Treated	Most	Elongated		Complete

Before Washing with PBS there are elongated and circular cells in the wells

Cells Form Strong Attachments with Fibronectin

After 2 h incubation- Washed

Condition	Cell Adhesion	Cell Morphology	Pattern	Extent of Spreading
Non-TC Treated	Few cells left	Circular	None	Not many cells
Half F_n Treated	Left side only			Most of cells left
Design drawn in F_n	On pattern	Mostly elongated, few circular		On the pattern drawn in F_n
F_n Treated	Completely	Elongated		Whole well

Washing left the strongly attached, elongated cells and very few circular cells

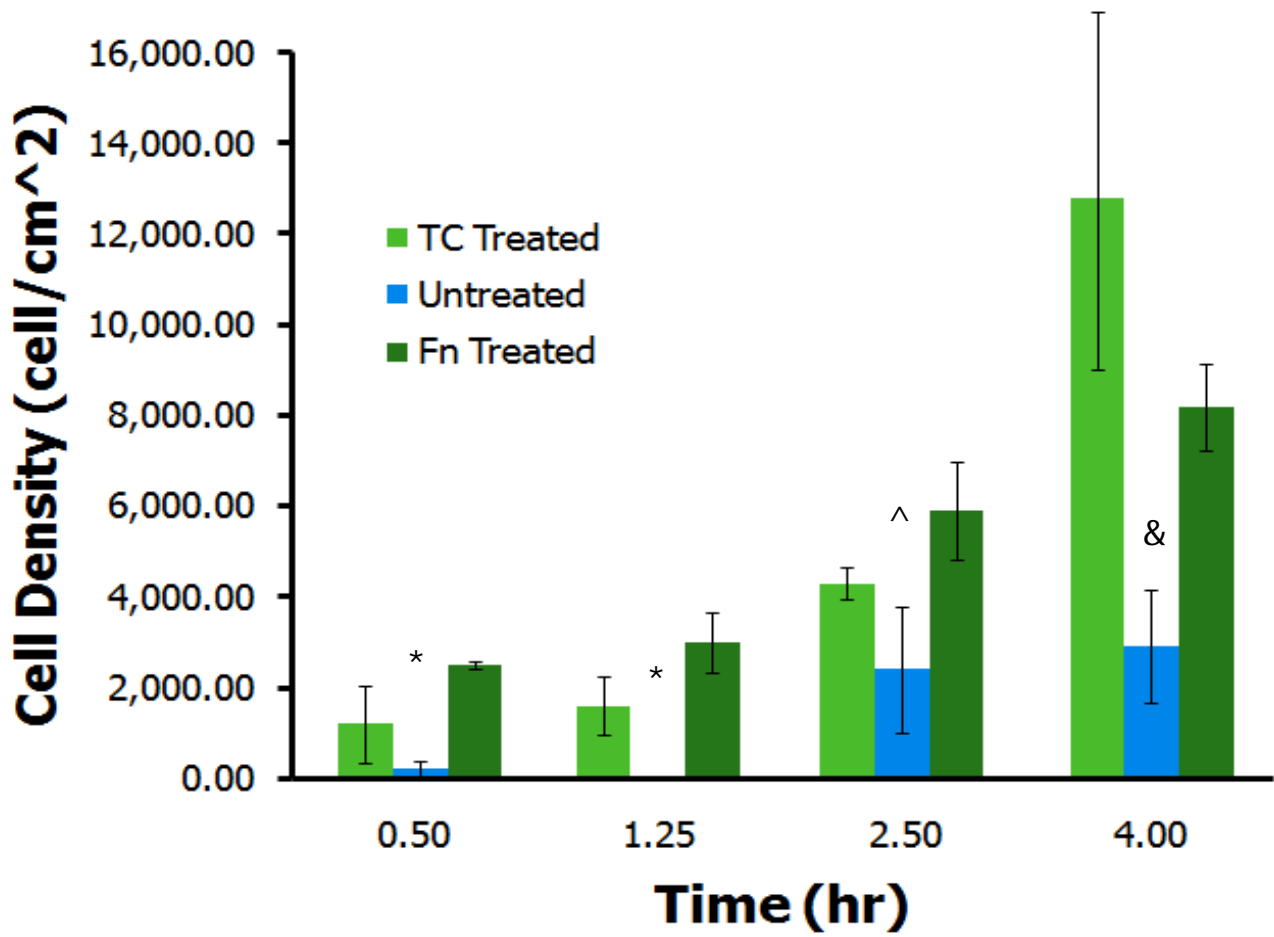


Fibronectin Promotes Cell Attachment



- Fibronectin is a primary mediator of cell surface attachment
 - Allows cells to bind to the surface
- Morphology shows that F_n allows cells to spread in 2 hours

More Cells Attach to F_n Coated and TC Plates Than UT Plates

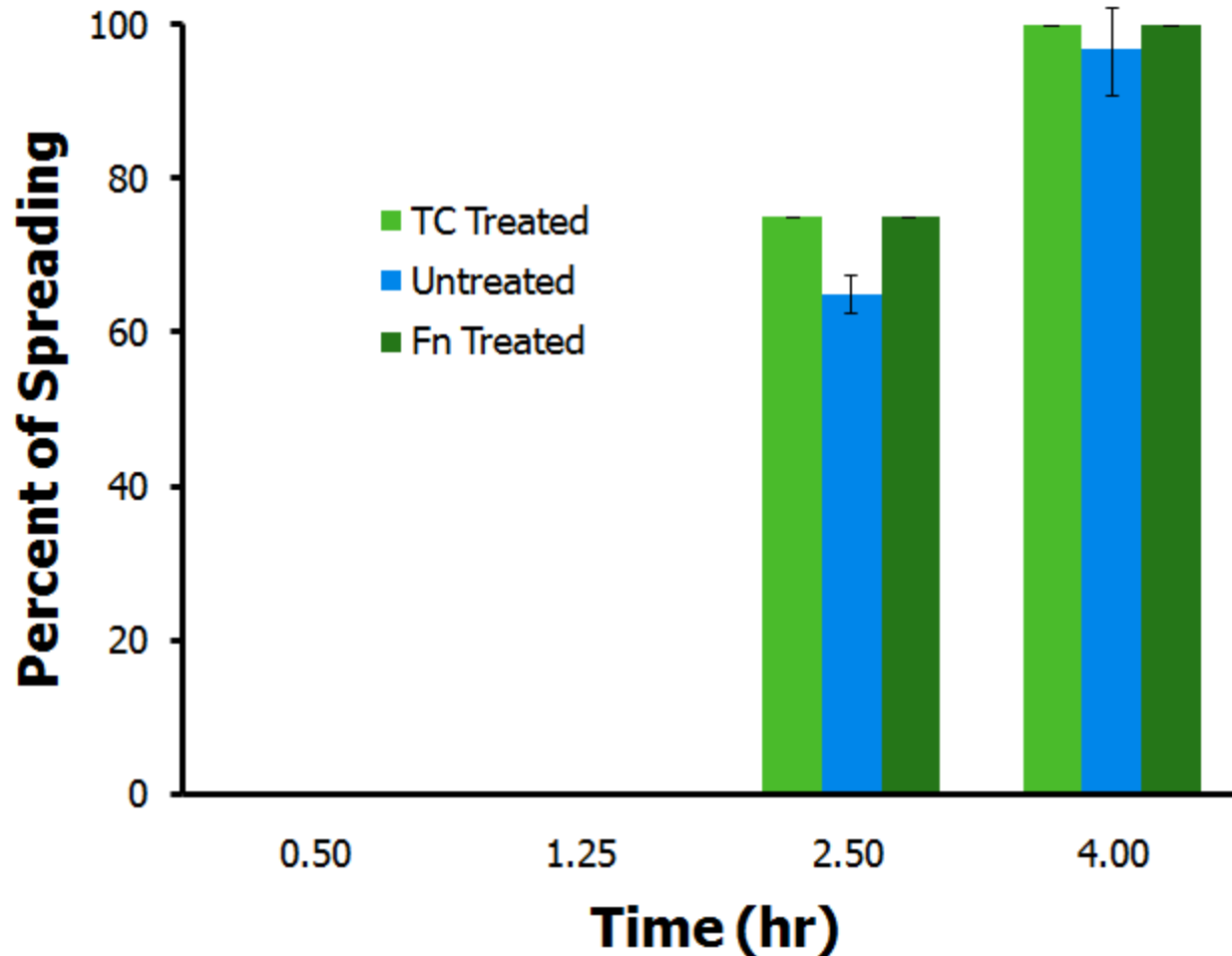


Single factor Anova shows difference

* Significant Tukey- all
^ TC vs UT not significant
& only TC vs UT significant

TC Treated & Fn Treated from XXXX's Data

No Significant Difference in Spreading Between Plates



Single factor Anova shows no difference



Significant Difference in Number But Not Spreading



- No significant difference in percent spreading between treatments
- Number of cells show significant differences between plates



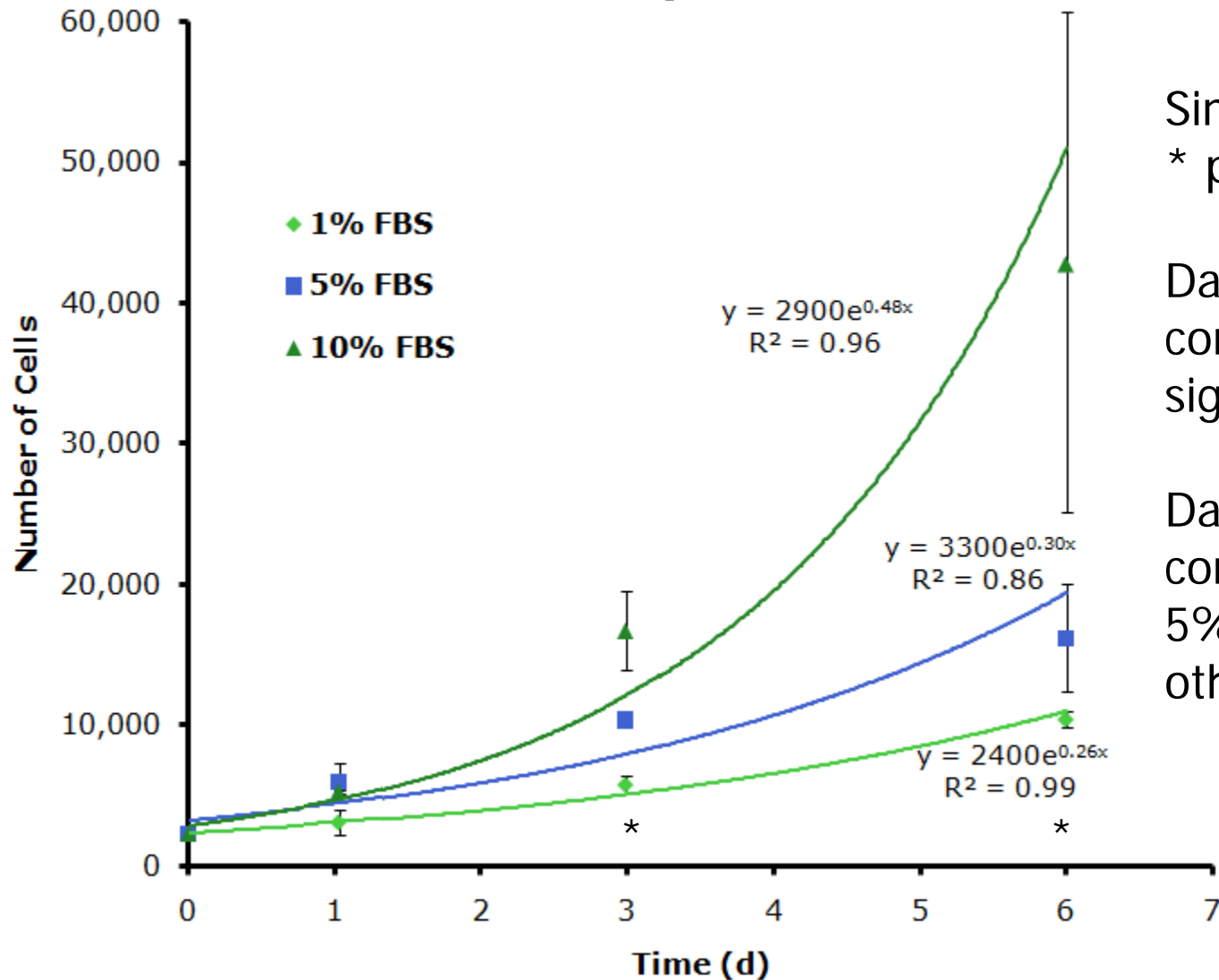
Two Experiments

Demonstrate HDF Attachment



- Different types of results
 - Quantitative Attachment Assay-quantitative data
 - F_n Attachment Assay-qualitative data
- Results agree
 - F_n promotes cell attachment and spreading as compared to no treatment

Proliferation Assay Cells Are in Exponential Growth




Single Factor ANOVA
* $p < 0.05$

Day 3: All Tukey
comparisons are
significant

Day 6: Tukey
comparison of 1% and
5% insignificant, all
others significant



Cells have Different Doubling Times in Different Media



- Cells in 1% FBS Media have longest doubling time (2.8 d)
 - 1% media has fewest nutrients
- Cells in 10% FBS have shortest doubling time (1.4 d)
 - 10% media has most nutrients
- Within the test conditions, more FBS translates to faster doubling times



HDF Cells Respond to Treated Surfaces and 10% FBS



- Treated and Fn coated plates
 - Significantly more attachment than untreated plates
 - No significant increase in % spreading from untreated plates
- 10% FBS media promotes cell proliferation more than 1% and 5% FBS media