

Attachment and Proliferation of Human Dermal Fibroblast (HDF) Cells in Culture

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Experimental Objectives

- To determine the effect of surface treatments, specifically tissue culture (TC) and fibronectin (Fn) treatments, on cell attachment
- To determine the effect of concentration of fetal bovine serum (FBS) in DMEM on cell proliferation.

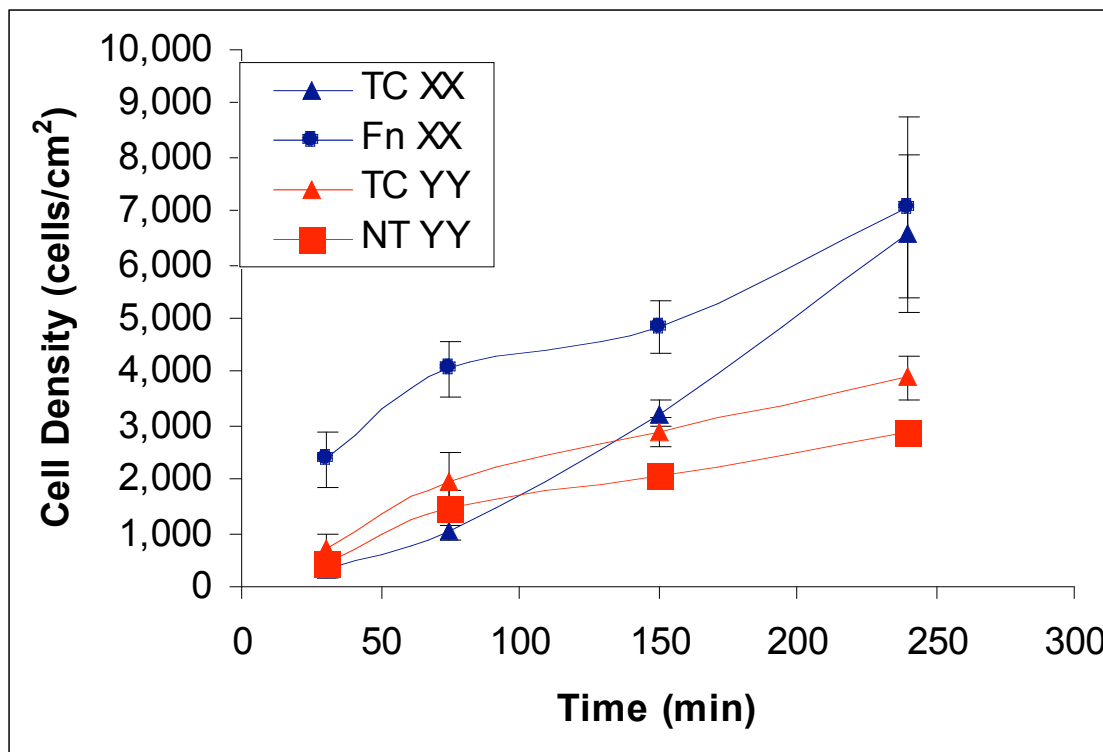
Quantitative Cell Attachment Assay

- Two comparative experiments– Untreated vs. TC-treated and TC-treated vs. Fn-Coated surfaces
- Three wells for each of four time points in each treatment: 30, 75, 150, and 240 minutes after seeding
- Wells were rinsed prior to counting to remove unattached cells
- Cells counted by light microscopy using a grid with area $.01 \text{ cm}^2$

Cell Proliferation Assay

- Three treatment groups – 1%, 5%, and 10% FBS in DMEM
- Three wells for each of four time points: 4 hours, 2 days, 5 days, and 7 days after seeding
- Cells cleaved from well with trypsin and counted with coulter counter

Observed Cell Attachment of Different Treatment Groups



- XX is investigator #1
- YY is investigator #2
- Fn is fibronectin coated
- TC is tissue culture treated
- NT is not treated

Differences in Growth at Final Time Point

- Two sample t-test assuming equal variance ($\alpha=0.05$) used to determine statistical significance
- Difference between Fn and TC treatment not significant at 4 hour time point ($p=0.53$)
- Difference between TC and no treatment is significant at 4 hour time point ($p=0.01$)

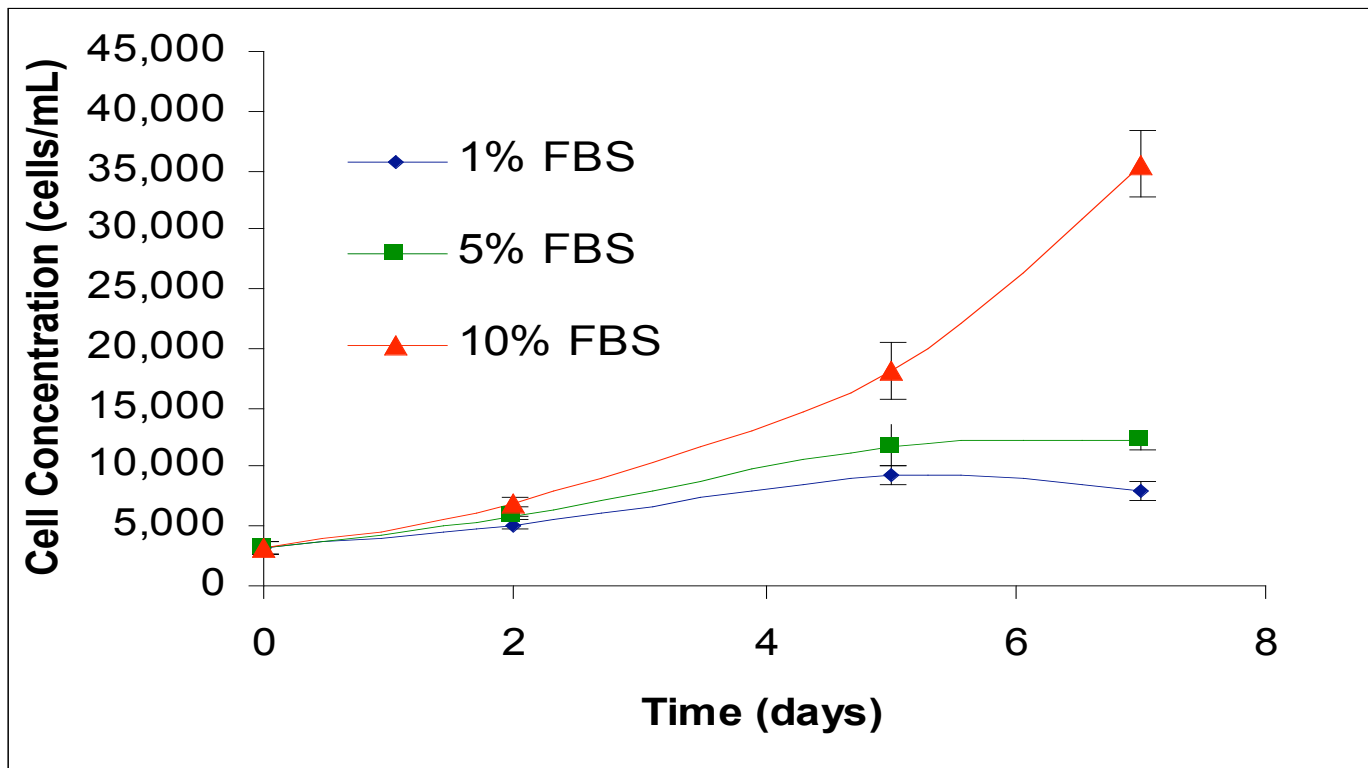
Differences in Rate of Growth Between Fn and TC treatment

- TC regression: $y = 20.9x + 2000$, $R^2 = 0.97$
- Fn regression: $y = 30.2x - 950$, $R^2 = 0.98$
- Even though final growth points are similar, rate of attachment over 4 hour time period in the Fn group is about 1.5 times greater than TC group

Fibronectin Attachment Assay: Fibronectin Encourages Cell Attachment

- Three wells for each of four treatment groups: no treatment, half Fn coated, X shaped coating, whole Fn coated
- Qualitatively assessed attachment after two hours by light microscope
- Cells observed to be larger and more spread in areas where well was treated with Fn
- Supports findings of quantitative assay

Cells Proliferate More Rapidly in Higher Serum Concentrations



Different serum concentrations have different proliferation rates

- Two sample t-test assuming equal variance ($\alpha=0.05$) used to determine statistical significance
- 1% and 5% ($p=0.002$), 5% and 10% ($p=0.006$), 1% and 10% ($p=0.004$) significantly different at day 7

Cell Doubling Time is Greater for Lower Serum Concentrations

- Used exponential fits to calculate, R^2 values shown in parentheses.
- 1%: 4.9 days ($R^2=0.83$)
- 5%: 3.5 days ($R^2=0.92$)
- 10%: 2.0 days ($R^2=0.99$)
- Decreasing R^2 value likely due to break in exponential growth → may indicate cells reach carrying capacity

Conclusions About Cell Culture Proliferation and Attachment

- As seen from day 7 comparison and cell doubling rates, cells proliferate at a higher rate in DMEM with 10% serum, followed by 5% and 1%.
- Attachment occurs faster with Fn treatment compared to TC treatment
- After four hours there is no significant difference in attachment between Fn and TC Treatment
- TC treatment increases attachment significantly compared to no treatment over four hours
- Fn increases cell attachment