Influence of Culture Environment on Human Dermal Fibroblasts (HDF) Attachment and Growth

YYY

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Objective

- To assess the effects of culture environment upon HDF in 24 well plates over time
  - To observe and quantify
    - HDF attachment on varying surfaces
    - HDF growth in DMEM with varying Fetal Bovine Serum concentrations

Assessment of HDF attachment to different surfaces

- Prepare 24 well plates with test conditions
  - **Tissue Culture (TC) treated**, **untreated**, and **fibronectin (Fn)-coated** surfaces
- Seed HDF grown in DMEM with 10% FBS
- Test conditions in triplicate at each time point
  - Qualitative assessment
    - After **2 hr** observe using light microscopy
  - Quantitative assessment
    - At **30 min**, **1 hr 15 min**, **2 hr 30 min**, and **4 hr** determine cell density using 10X objective on light microscope

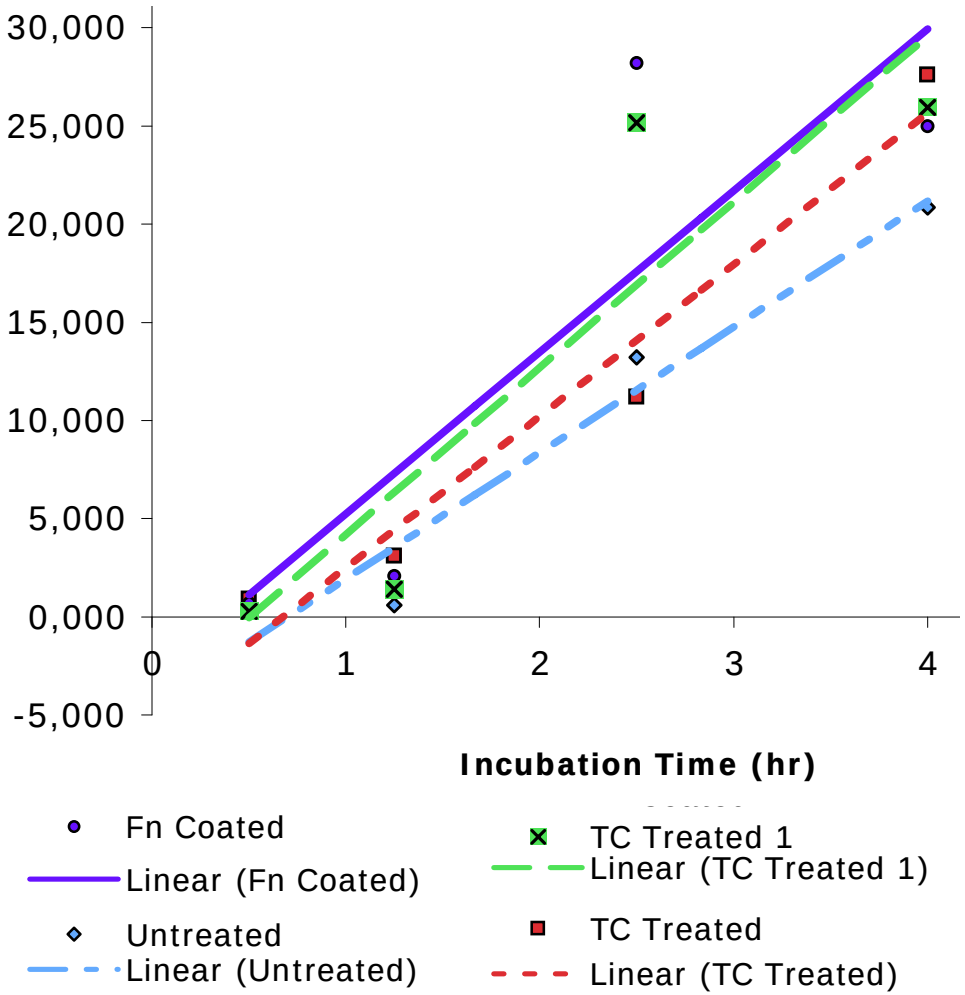
More HDF Attach to Fn-Coated than to TC-Treated Surface within 2 hours

- Cell density is greater for Fn-coated surfaces than TC-treated surface in 2 hours
  - Painted Fn designs were visibly denser with HDF than TC background
  - Wells half painted with Fn was also visibly denser and shared a vague border with less dense TC-treated half

Pictures here

- More HDF on Fn surfaces have begun extending

HDF Attach to Test Surfaces at Different Rates



- Rates of cell density of from high to low:
  - HDF on Fn coated, TC treated, then untreated
- Cell density vs. time reach a plateau
  - At ~2-3 hr for Fn coated and TC treated
- More incubation time generally means more cells are attached
  - ~4 hours enough time for most cells to attach

(Untreated and TC Treated 1 are XXX data)

HDF Attach More Sufficiently to Fn and TC treated surfaces

- Qualitative assessment at 2 hours
  - Difference in HDF's affinity for Fn-coated and TC-treated surfaces
- Quantitative assessment at 30 min to 4 hours
  - Contrast between cell density on Fn-coated, TC-treated, and untreated surfaces
  - Differences appear more distinct earlier (~30 min-2 hours), as already shown in qualitative assessment
- With more incubation, a greater percentage of cells seeded attach to surfaces
  - A plateau is reached in cell density rate where almost all cells have attached within 4 hours for Fn-coated and TC-treated

Assessment of HDF growth in response to varying % FBS

- Seed HDF into TC-Treated 24 well plates under test conditions
  - DMEM with **1%**, **5%**, and **10% FBS**
- Test conditions at each time point
  - Anti-PCNA
    - Stain a well at each condition and 3 wells with HDF in DMEM with 10% FBS as control after **2 days**
    - Observe under a light microscope
  - Cell Proliferation Assay
    - Determine cell density using light microscope and Coulter count triplicates of each condition after **1**, **3**, and **6 days**
    - Coulter Count cells attached after **4 hours** as control and initial cell count

% FBS Affects Percentage of Cells Undergoing S-phase

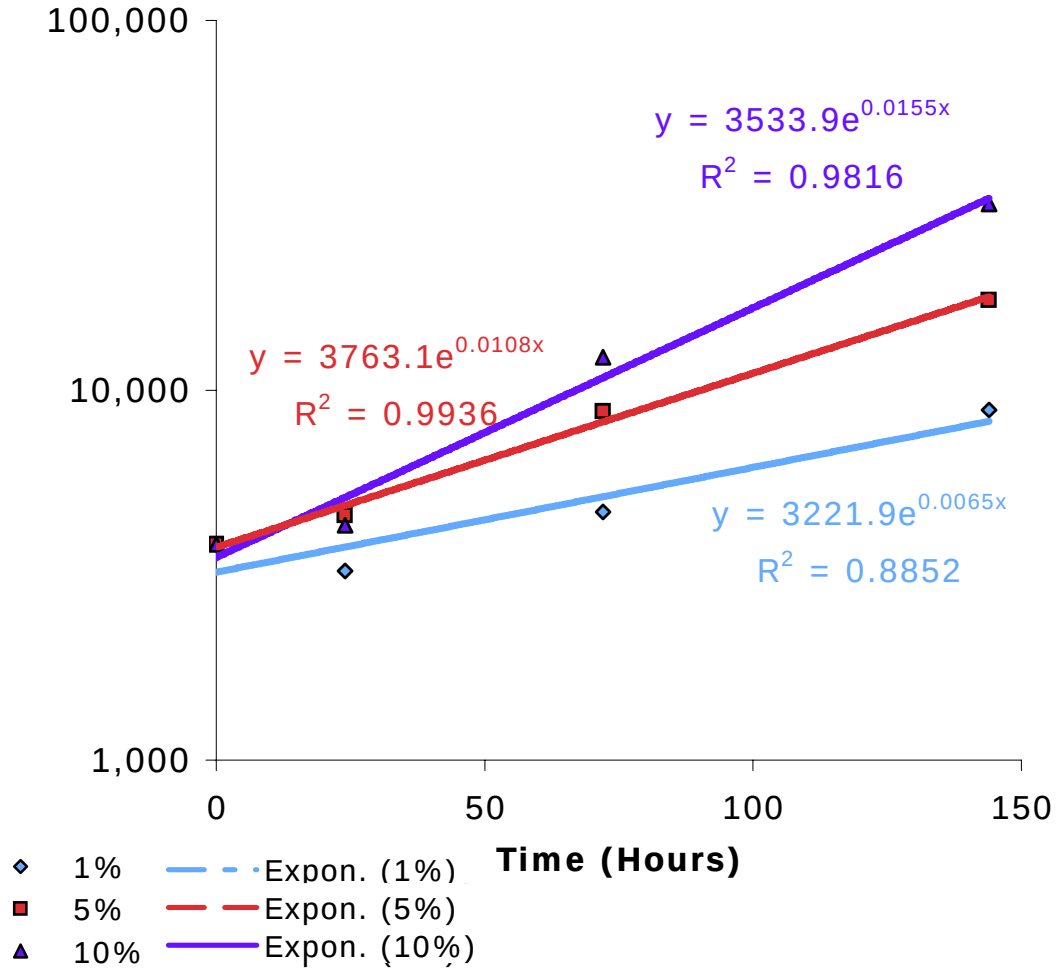
- Anti-PCNA staining stained nuclei undergoing S-phase red

Picture here

- After 2 days of incubation, wells with greater % serum had greater % of cells undergoing S-phase

% Serum	% S-phase
1%	48.40%
5%	59.10%
10%	72.90%

Higher % FBS Correlates with Higher Growth Rate



- HDF growth is exponential
  - R^2 values are above 0.8 for exp. fit
- Cells/well is different between FBS concentrations
  - ANOVA Tukey test resulted in $p<0.05$
- Lag time appears to be ~24hr
  - Affects HDF in 1% FBS the most

Up to 10% FBS is Conducive to HDF Growth

- After seeding cells:

	After 2 days	After 3 days
% Serum	% S-phase	Cells/well
1%	48.40%	4690
5%	59.10%	8780
10%	72.90%	12300

- HDF in S-phase continue on through cell cycle to undergo mitosis
  - Increased S-phase correlates with increased HDF growth rate
- Both anti-PCNA staining and cell proliferation assay show that HDF proliferation is higher in higher concentrations of FBS for 1%, 5%, and 10% FBS in DMEM

Culture Environment Affects HDF Behavior

- Effect of surface
  - Fn-coated and TC-treated aid cells to attach quicker by providing an initial anchor
  - Untreated requires proteins in media and from cells to deposit first before cell attachment
- Effect of media
  - Higher serum concentration provides nutrients for quicker cell proliferation
    - This leads to higher growth rate
- Attachment time affects initial HDF growth on new surfaces