An Analysis of Fibronectin and Serum on the Attachment and Proliferation of HDF Cells

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HDF Cell Analysis Objective

- To qualitatively analyze human dermal fibroblast (HDF) cell attachment to different plate surfaces
  - Fibronectin Attachment Assay
- To quantitatively assess the proliferation and cell cycle stage of HDF cells within different media conditions
  - Anti-PCNA Staining
- To quantitatively evaluate the degree of growth and replication of HDF cells as a result of different serum concentrations
  - Cell Proliferation Assay

Evaluating HDF Attachment on Different Surfaces

- Fibronectin (Fn) Attachment Assay
  - Non-TC-treated well plates were prepared with three different test conditions and a control:
    - Half Fn and half non-TC-treated surface
    - Fn design on non-TC-treated surface
    - Fn coated surface
    - No surface coating (control)
  - HDF cells were seeded onto the plate at a uniform concentration of 50,000 cells/mL with DMEM and incubated for 2 hours.
  - Cell adhesion and morphology were observed with a light microscope.

Assessing Cell Cycle Stage with Different Media Conditions

- Anti-PCNA Staining
  - HDF cells were seeded at a uniform concentration of 20,000 cells/mL in three test conditions and incubated for 2 days.
    - Test conditions: DMEM with 1, 5, and 10% fetal bovine serum (FBS) and 1% antibiotic
  - Anti-PCNA-mouse IgG antibody and Anti-mouse IgG-Horse Radish Peroxidase antibody were used in the standard cell staining procedure.
  - Cell staining was visualized with a light microscope to determine the percentage of cells in S-phase.

Measuring Rate of Cell Proliferation

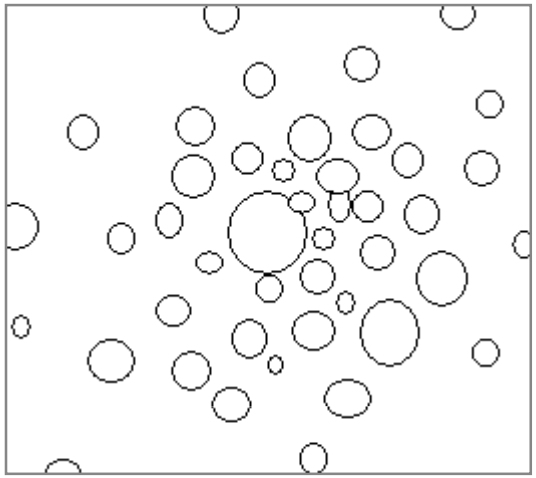
- Cell Proliferation Assay
  - HDF cells were seeded at a uniform concentration of 5,000 cells/mL in three test conditions
    - Test conditions: DMEM with 1, 5, and 10% FBS and 1% antibody.
  - Each test condition was incubated for 24, 72, and 168 hours.
  - Cell growth was assessed with a Coulter Counter to count the number of cells in each media condition and time point.

Fn Allows Cells to Adhere to the Plate Surface

	Areas with Fn	Areas without Fn
Cell Adhesion	Strongly attached with extended pseudopodia, a few unattached cells	Not strongly attached, if at all
Cell Morphology	Elongated, pseudopodia extended in multiple directions, a few round cells dispersed	Round
Cell Spreading	Evenly dispersed aside from a few large cell clumps	Some clustering, especially in the middle of the well due to media movement

Surface Comparison in Areas with and without Fn

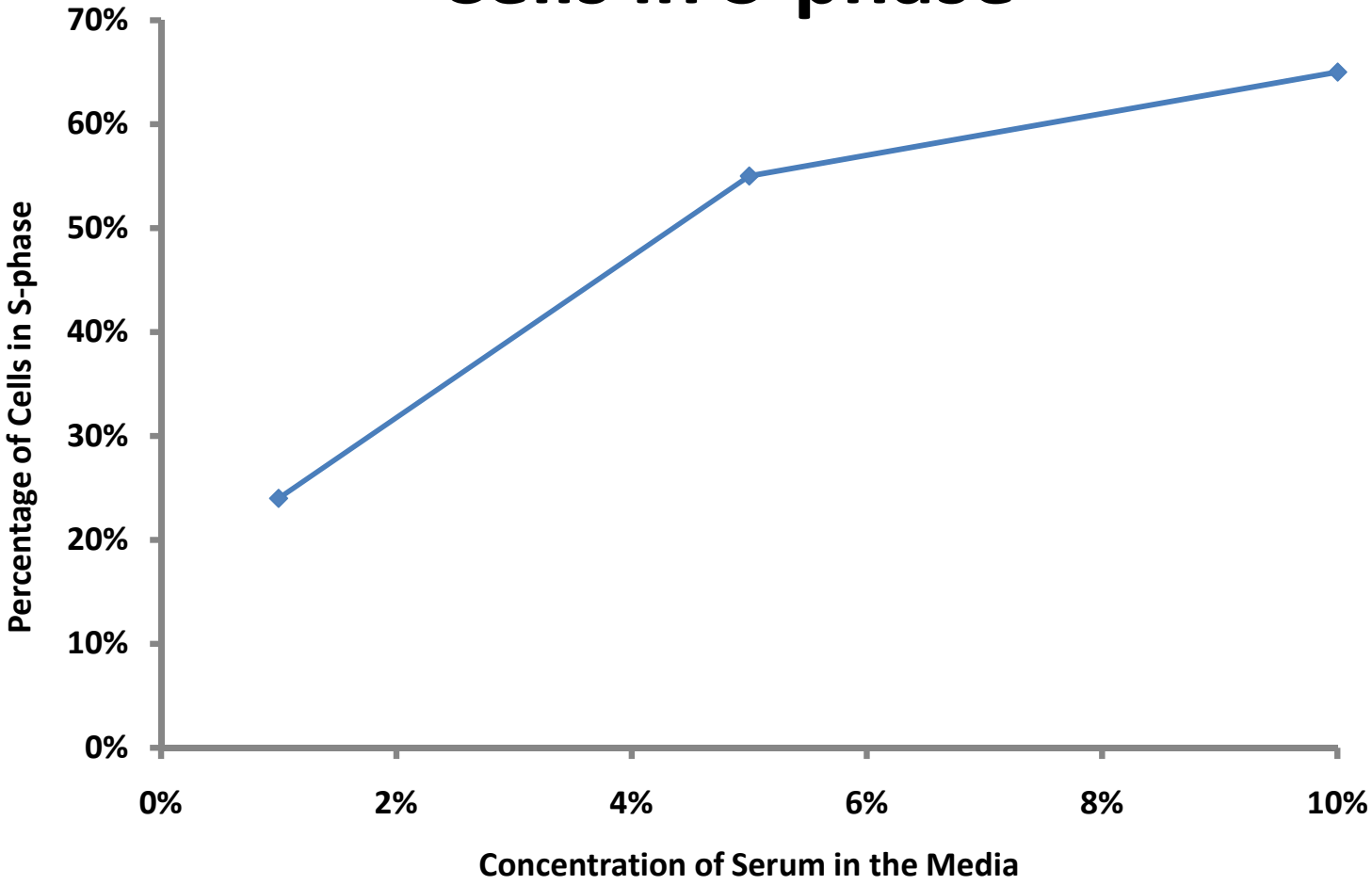
Cells on Fn Treated Surface



Cells on Non-TC-Treated Surface

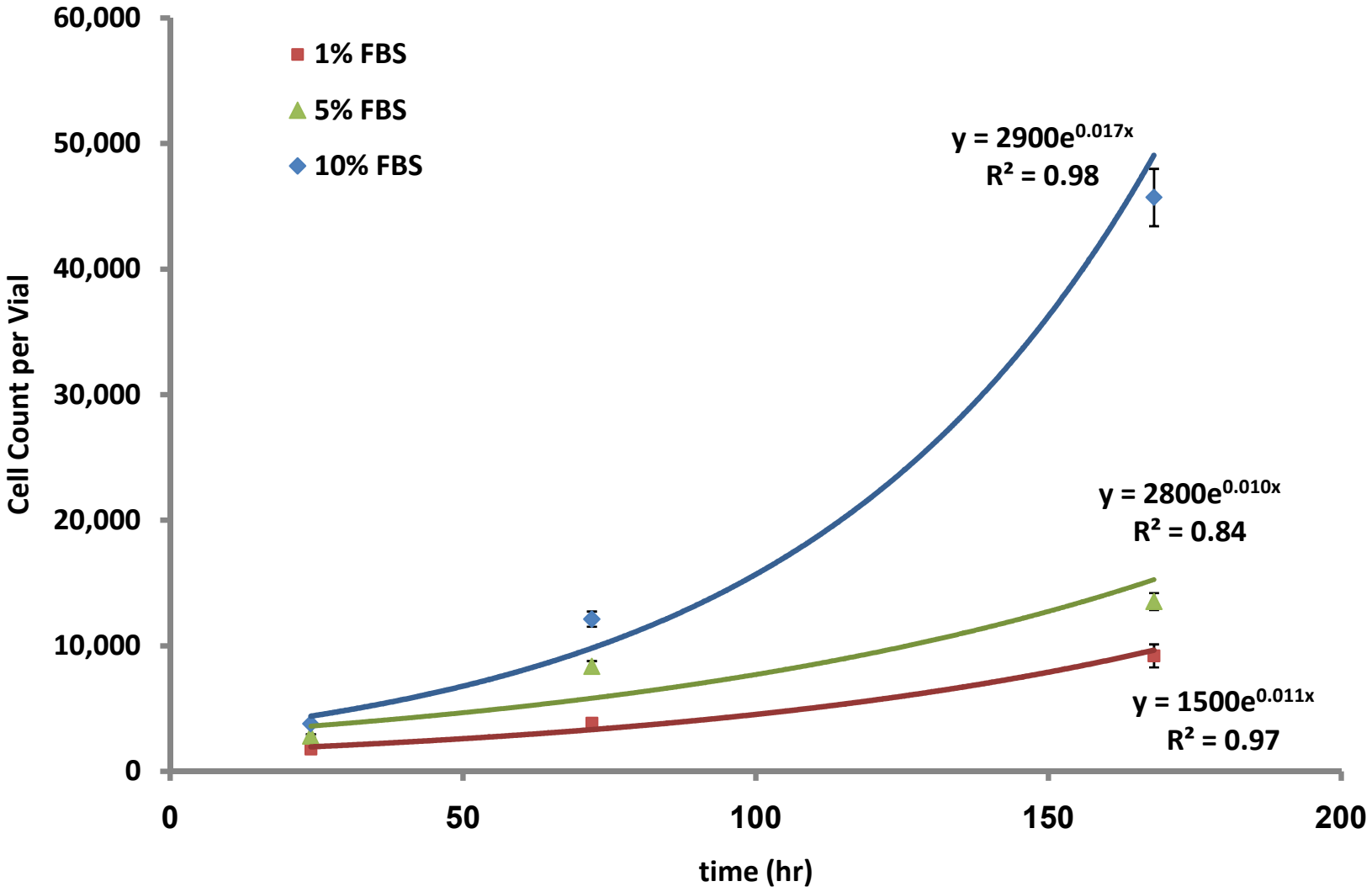
- Clear distinction between surfaces with and without Fn in the wells
  - Designs within the wells were marked by the distinct cell morphology on the left
  - Areas without Fn looked similar to the picture on the right, distinctly different from areas with Fn
- The cells adhered only to the area where Fn was painted on the surface, allowing the cells to grow and take shape.

FBS Results in a Greater Percentage of Cells in S-phase



- As the FBS concentration increases, the percentage of cells in S-phase increases, indicating a possible increase in the number of dividing cells.

Cell Proliferation with Different Media Conditions



FBS Increases Cell Growth Over Time

- As the percentage of FBS increases, the cell growth increases exponentially, indicating increased cell replication.
- The means for each time point and media condition have a statistically significant difference verified by ANOVA with Tukey's post-hoc ($p<0.05$).

Comparison of Cell Proliferation Experiments

	Anti-PCNA Staining	Cell Proliferation Assay
Test Conditions	HDF cells in DMEM with 1, 5, 10% FBS	HDF cells in DMEM with 1, 5, 10% FBS and 24, 72, 168 hour incubation times
Type of Data	Cells stained red or blue according to whether they are in the S-phase of division	Cell counts for each test condition
Results	An increase in FBS concentration resulted in an increase in the percentage of cells in S-phase.	An increase in FBS concentration resulted in increased exponential cell growth.

Analysis: FBS results in more cells in S-phase in the Anti-PCNA Staining, indicating that more cells are entering into division and therefore there is more cell growth, in line with the Cell Proliferation Assay.

HDF Cell Culture Facilitated by Fn and FBS

- Fibronectin
  - Allows HDF cells to bind more frequently
  - Promotes cell elongation, cell spreading, and pseudopodia extension

  → Fn is binds to integrins (or receptors) on the cell surface to aid the specific cell substrate interaction, which increases cell adhesion.[1]
- Fetal Bovine Serum
  - Provides nutrients to the cells to allow for a greater percentage of cells in S-phase
  - Over time, aids the cells to exhibit an exponential increase in cell growth due to the initial lag time, also due to an increase in nutrient availability

  → Serum contains growth factors, adhesion factors, minerals, lipids and hormones and other nutrients that nourish the cells, aiding in proliferation and attachment. [1]

References

1. Freshney, R.I. Culture of Animal Cells. Ann Arbor: National Archive Publishing Company, 2008.