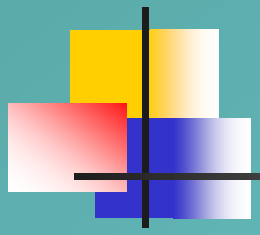
Human Dermal Fibroblast Attachment and Proliferation Assays

BIOE 342 Section 3

Purpose of Cell Attachment and Proliferation Assays

- Objectives
  - To qualitatively assess the attachment of human dermal fibroblast (HDF) cells to fibronectin (Fn) and untreated polystyrene
  - To observe how cell cycle and proliferation of HDF cells are affected by serum conditions
  - To compare and contrast results from qualitative and quantitative cell proliferation assays

Qualitative Cell Attachment Assay Methods



- Use a non-TC (Tissue Culture)-treated well plate.
- Add phosphate buffered saline (PBS) to 3 control wells (Condition A), paint Fn on half of 3 wells (B), draw an “X” pattern with Fn on 3 wells (C), and add Fn to 3 wells covering entire bottom of well (D).
- Determine cell concentration of HDF cells provided with Coulter Counter and dilute to 50,000 cells/mL.
- Seed cells on 12 wells and incubate for 2 hours.
- Use light microscope and record pattern of attached cells and extent of spreading.
- Aspirate supernatant, add PBS, and use light microscope again.
- Record pattern of attached cells and extent of spreading.

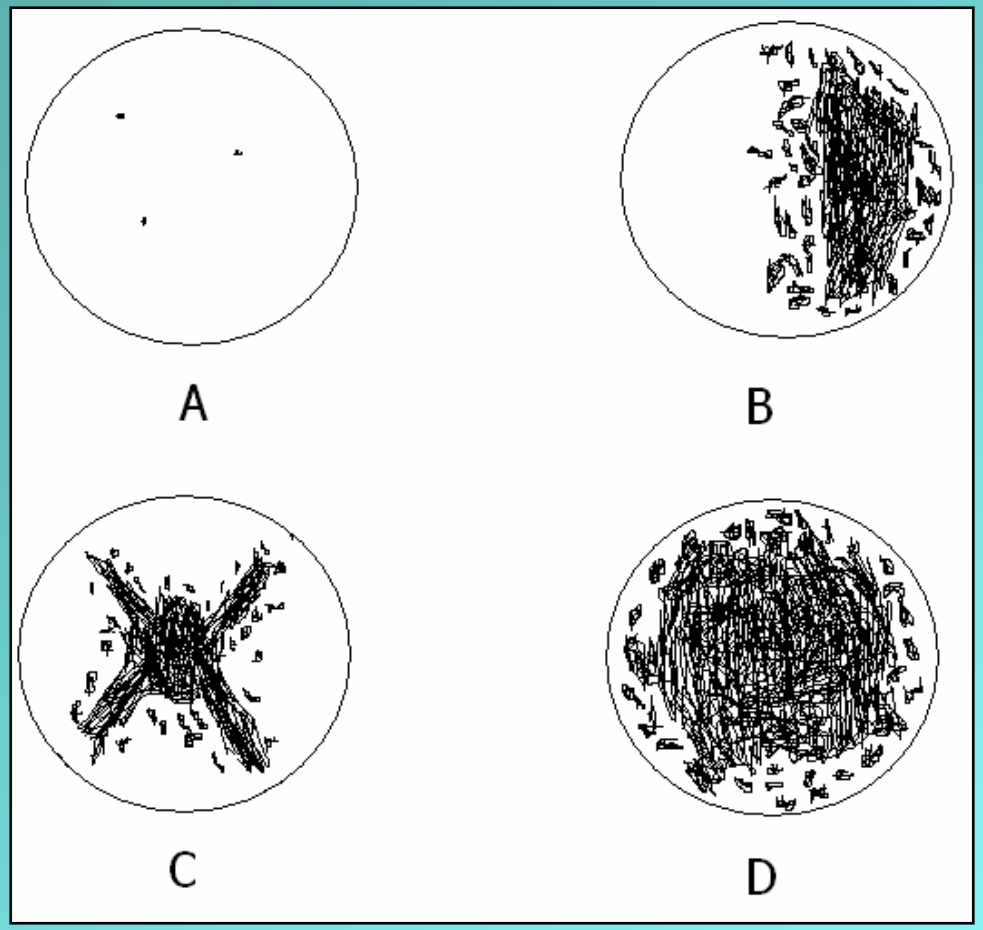
Qualitative Cell Proliferation and Cell Cycle Assay Methods

- Prepare 3 cell suspensions at 20,000 cells/mL of HDF cells of 1, 5, and 10% fetal bovine serum (FBS) (test conditions) each with DMEM and 1% antibiotics.
- Seed 1 well for each FBS condition and 3 wells with only DMEM, 10% FBS, and 1% antibiotics (controls). Incubate for 2 days.
- Wash wells and add formalin to fix cells.
- Wash wells and add H_2O_2 to eliminate peroxidase interference.
- Wash wells and add Anti-PCNA (Proliferating Cell Nuclear Antigen) primary antibody with Horse Radish Peroxidase (HRP) tag to 3 test conditions and control 1.
- Wash wells and add Anti-mouse IgG (Immunoglobulin G) secondary antibody to 3 test conditions and control 2.
- Wash wells and add AEC dye to stain nuclei that contain HRP red.
- Add hematoxylin dye to stain all live cells blue.
- View cells with light microscope and record observations of morphology and the color of cells and nuclei.

Quantitative Cell Proliferation Assay Methods

- Prepare cell suspension at 5,000 cells/mL of HDF cells with DMEM, 1% FBS, and 1% antibiotics.
- Seed 6 wells for Day 0 and 27 wells for Days 2, 5, and 7 with 1% FBS suspension.
- Incubate for 4 hours. Use Coulter Counter to determine Day 0 cell concentrations.
- Replace the media for Days 2, 5, and 7 with 3 wells each of 1, 5, and 10% FBS (test conditions) with DMEM and 1% antibiotics.
- Determine cell concentrations for Days 2, 5, and 7 on their respective days using the Coulter Counter.
- Replenish media with corresponding FBS conditions on Days 2 and 5 for cells that have not been counted yet.

Qualitative Cell Attachment Assay Data and Results



HDF cells are modeled as black objects

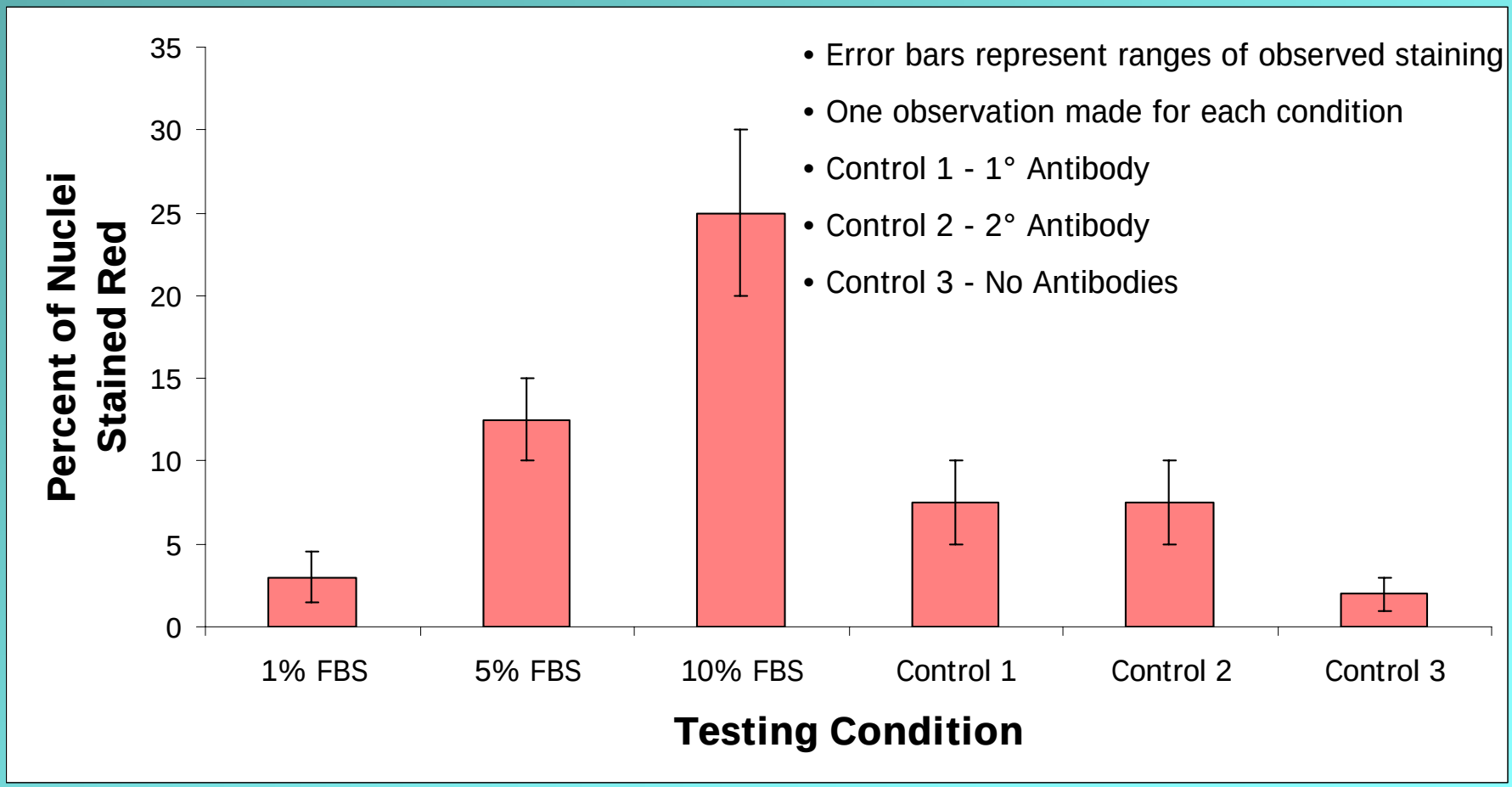
Test Conditions

- Well A – control (no Fn)
- Well B – half control / half Fn
- Well C – “X” of Fn
- Well D – Fn-soaked bottom

Results

- HDF cells attach more readily in the presence of Fn than untreated polystyrene.
- Elongation and pseudopodia formation of HDF cells are facilitated by Fn.

Qualitative Cell Proliferation and Cell Cycle Assay Data



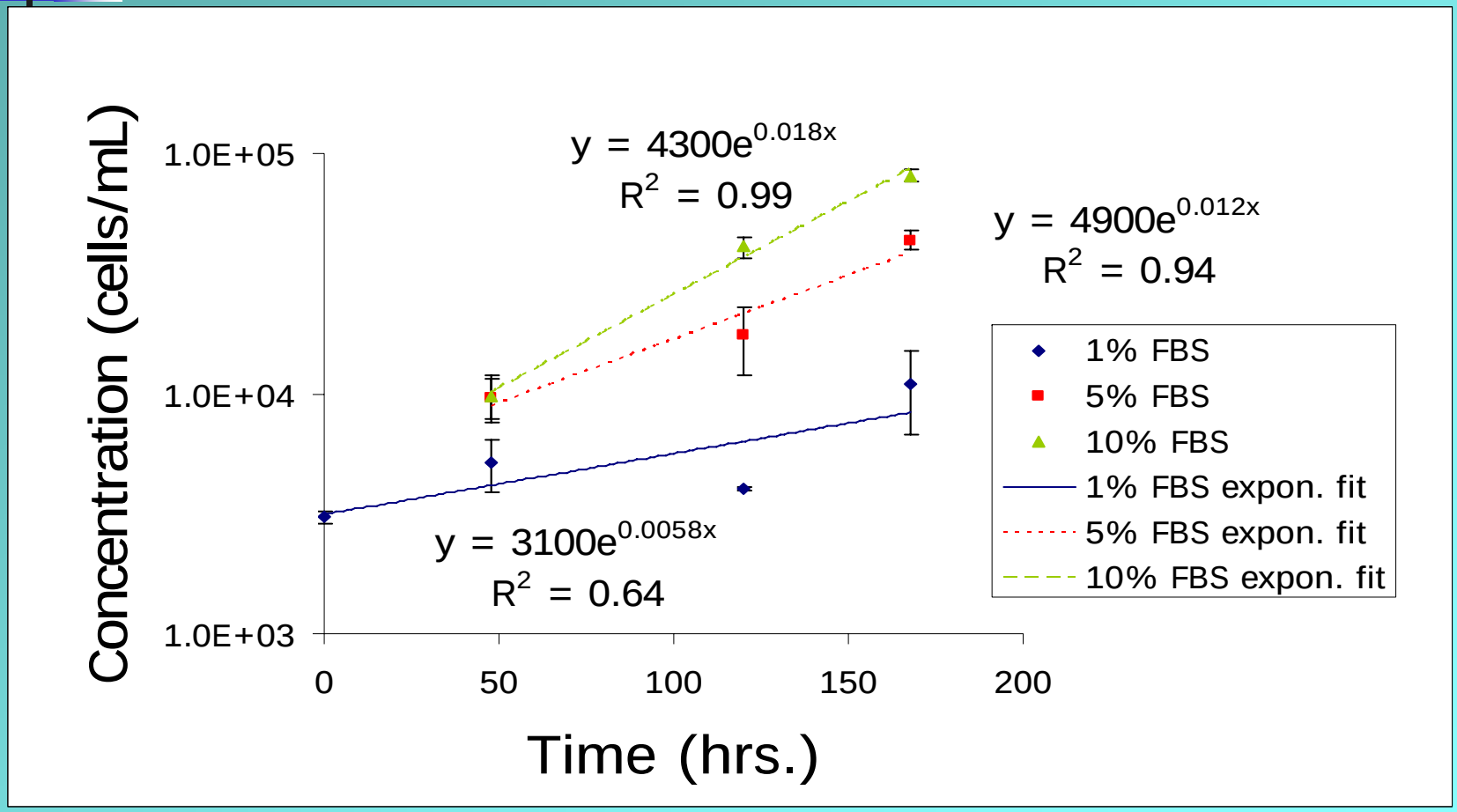
Qualitative Cell Proliferation and Cell Cycle Assay Results

- Morphology and Color of HDF cells
  - All cells stained blue – all cells were alive
  - Most cells were elongated with pseudopodia
  - Some cells with red-stained nuclei were round – cells are round during mitosis and cell division
- Control wells 1 and 2 stained more than predicted.
  - Control 1
    - Incomplete elimination of peroxidase activity by H_2O_2; AEC cleaved without 2° antibody present
    - H_2O_2 may need to be replaced
  - Control 2
    - Nonspecific binding of 2° antibody
    - Reduce 2° antibody incubation time with cells

Qualitative Cell Proliferation and Cell Cycle Assay Results

- 10% FBS gave the highest percentage of HDF cell nuclei stained red.
  - Highest percentage of cells in S-phase of cell cycle
  - Correlates to proliferation rate: if more cells are proliferating, more cells are likely to be in S-phase
- Percentage FBS positively affects percentage of HDF cells in S-phase.
  - A higher percentage of cells proliferating causes a higher percentage of cells to be in S-phase.
  - FBS provides agents that are desirable for HDF cell proliferation and division.

Quantitative Cell Proliferation Assay Data



Error bars represent standard deviations. n=3 for all readings except 0 hrs. (n=5).

Quantitative Cell Proliferation Assay Results

- HDF cell proliferation fits exponential growth model well for 5 (R^2=0.94) and 10% (R^2=0.99) FBS.
  - 1% FBS cells were not growing exponentially (R^2=0.64) – lacked agents from FBS that are desirable for HDF cell proliferation
- One-tail t-test assuming equal variances, $p<0.05$
  - 10% FBS, significant statistical difference of concentrations between Days 2 and 5 (p=1.6E-4), 2 and 7 (p=1.0E-5), and 5 and 7 (p=2.1E-4)
  - Day 7, significant statistical difference of concentrations between 1 and 5 (p=3.0E-4), 1 and 10 (p=2.3E-5), and 5 and 10% (p=2.6E-4) FBS
- Doubling times for HDF cells: 120, 58, and 39 hours for 1, 5, and 10% FBS, respectively
- FBS provides agents that are desirable for increasing HDF cell proliferation rate, reaching exponential growth.
  - 10% FBS proliferated exponentially, fastest.
  - 1% FBS proliferated the slowest, did not grow exponentially
  - 5% FBS reached exponential growth, slightly slower than 10%

Comparison and Contrast of Cell Proliferation Assays

- Both proliferation assays suggest a positive relationship between HDF proliferation and FBS from 1 to 10% FBS
- Both – 1% FBS does not increase HDF proliferation; both – 5 and 10% FBS do increase HDF proliferation
- Quantitative – HDF proliferation/FBS relation directly and fit an exponential growth model; qualitative – HDF proliferation/FBS relation indirectly through number of cells in S-phase;
- Quantitative – only considered cell concentration; qualitative – considered morphology to look for rounded cells participating in cell division
- Quantitative – used statistical analysis to determine strength of HDF proliferation/FBS relation (strong argument); qualitative – human opinion on relative amount of staining for indirect HDF proliferation/FBS relation (weak argument)
- Quantitative – concentrations measured over one week period to find actual change in cell number with time (HDF proliferation rate); qualitative – found percentage of HDF nuclei stained red at one time point (cannot use one time point to calculate rate)

HDF Cell Attachment and Proliferation Assays Summary

- HDF cells
  - Attach more readily to Fn than to untreated polystyrene
  - Elongate and develop more pseudopodia with Fn than with untreated polystyrene
  - Change morphology (elongated to round) during cell division
  - Are more likely to be in S-phase of cell cycle in 10% FBS than 1 or 5% FBS
  - Proliferate exponentially in 5 and 10% FBS over a 7 day period *in vitro*
  - Proliferate at different rates in 1, 5, and 10% FBS – 10% being the highest and 1% being the lowest
- Qualitative cellular assays can be used to find a correlation.
- Quantitative cellular assays can be used to find if a correlation is due to a causation based on statistical analysis and mathematical modeling