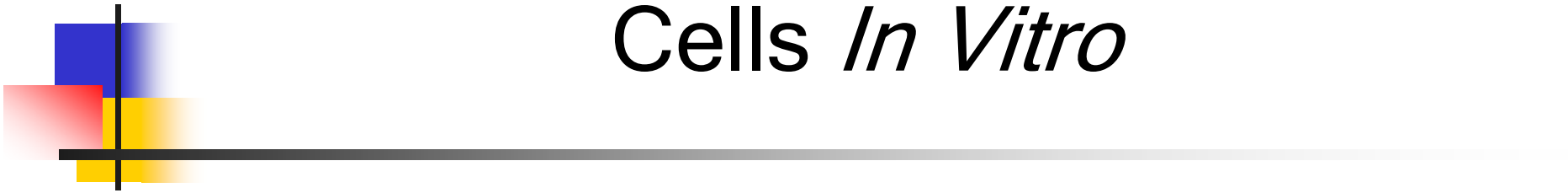
Proliferation and Viability of Human Dermal Fibroblasts (HDF) Cells *In Vitro*



Rice University

Objectives – HDF Cell Proliferation

- To quantitatively assess the effects of serum on the growth and replication of HDF cells
- To quantitatively correlate the rate of cell proliferation and the cell cycle through antibody-specific staining procedures

Objectives – HDF Cell Viability

- To assess the impact of toxic material on the viability of living cells
- To analyze the effect of fluorescent dye staining

Experimental Methods – Cell Proliferation Assay

- HDF cells seeded into 24-well plate at 5,000 cells/mL and incubated for seven days
- Cells fed with DMEM containing 1%, 5%, or 10% Fetal Bovine Serum (FBS) for Day 0, 2, 5, and 7 treatments
- Cell density determined using Coulter Counter

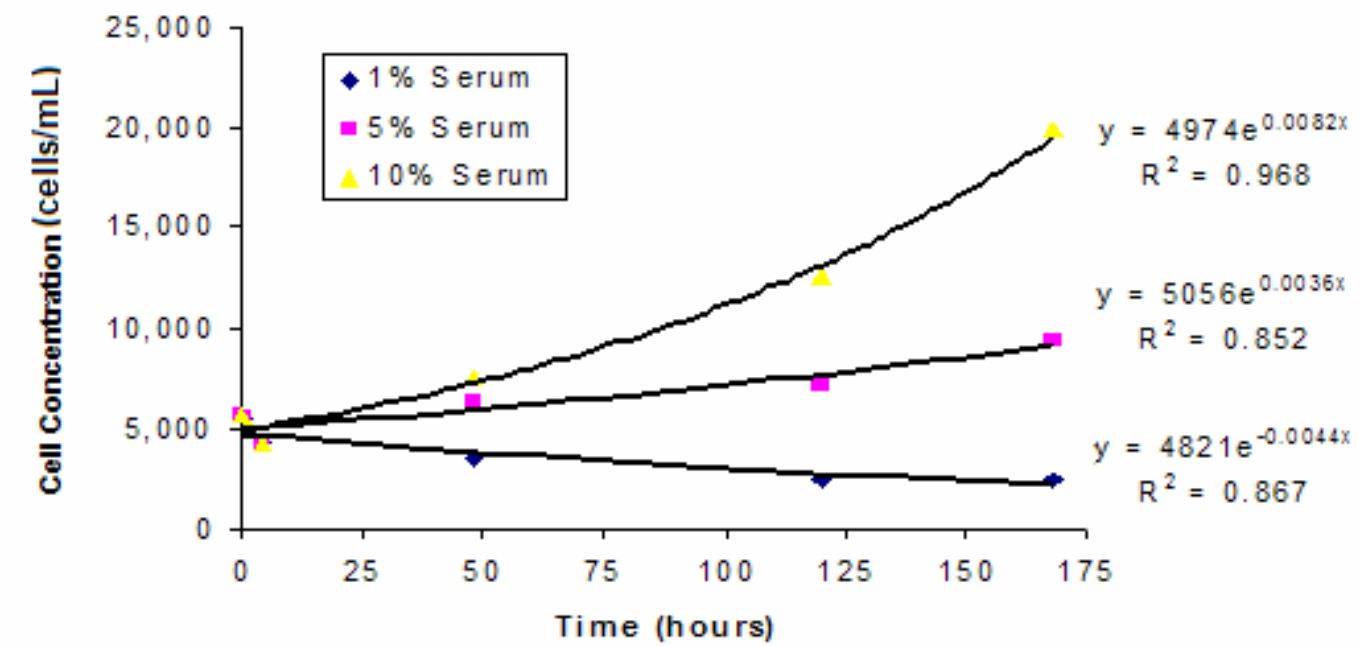
Experimental Methods – Anti-PCNA Staining

- HDF cells seeded into 24-well plate at 20,000 cells/mL and incubated over 48 hours in DMEM containing 1%, 5%, or 10% FBS
- Cells stained with Anti-PCNA primary antibody, Anti-Mouse IgG secondary antibody, AEC solution, and hematoxylin
- Control wells were stained with only primary, secondary, or neither antibodies
- Presence of stained nuclei quantitatively and qualitatively analyzed using light microscope

Experimental Methods – Live/Dead Fluorescence Assay

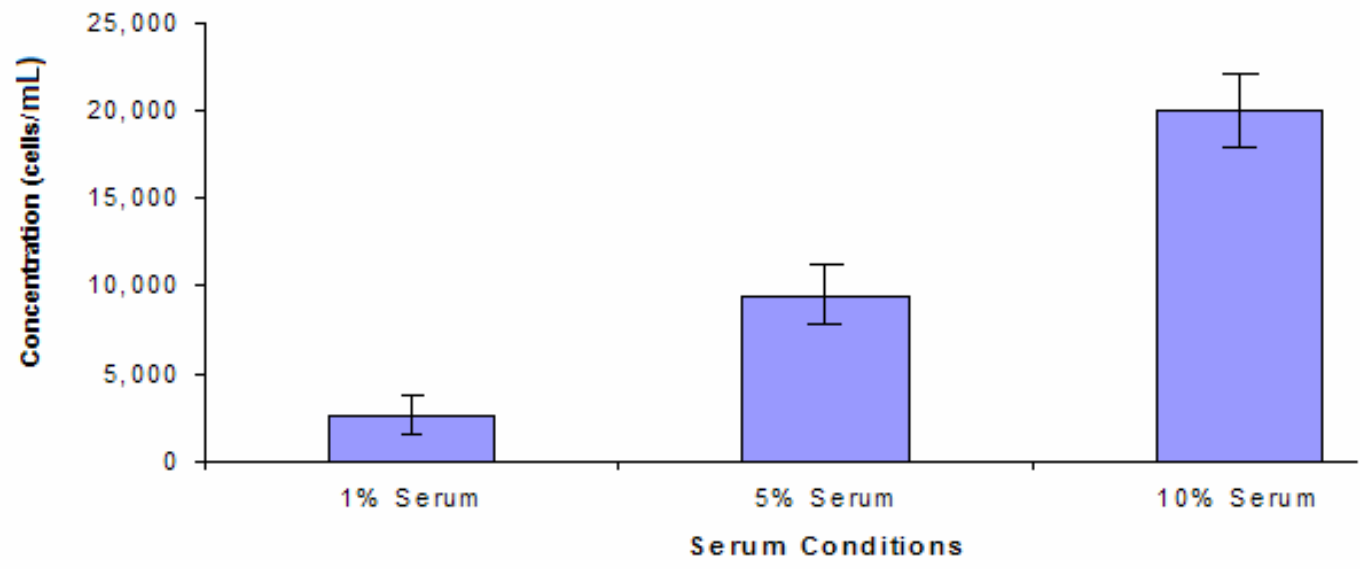
- HDF cells seeded in 24-well plate and incubated for 48 hours
- Cells treated with PBS, ethanol, or 2 drops of ethanol and EthD-1 fluorescent dye and incubated for 30 minutes
- Cells qualitatively analyzed using fluorescence microscope

Cell Proliferation of HDF Cells in Different Serum Conditions



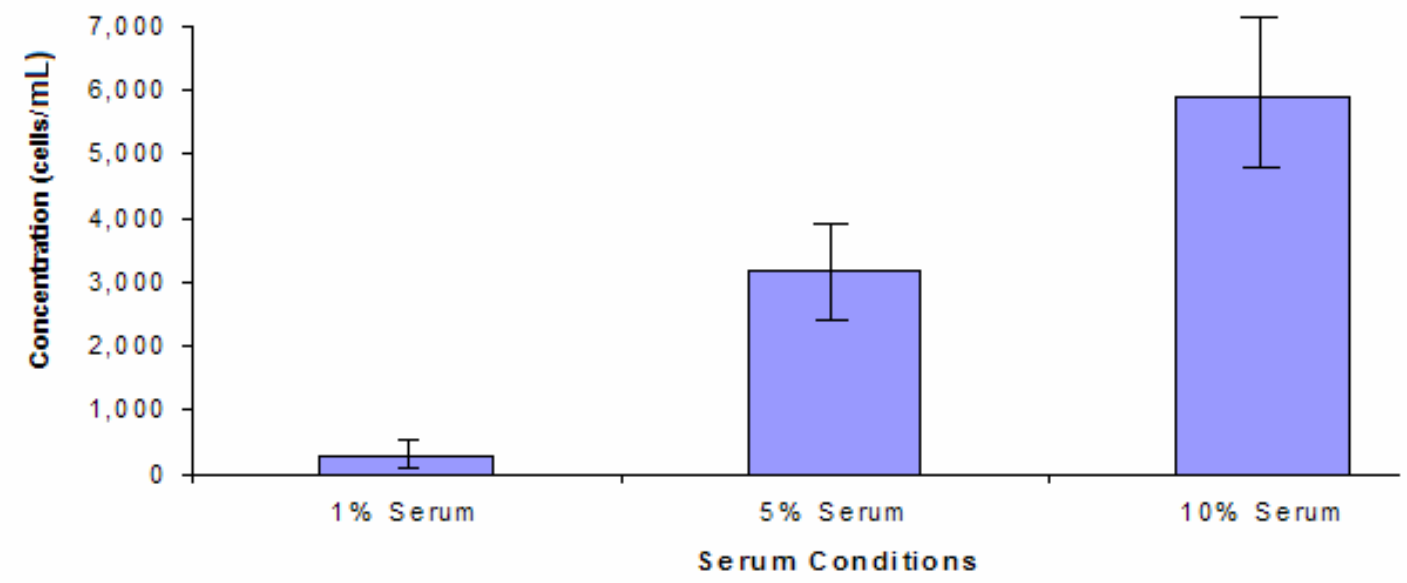
10% serum concentration produced a greater HDF cell growth rate relative to 1% and 5% serum concentrations (p-value < .01)

Differences in Cell Density on Day 7



Cell density was significantly greater for cells grown in 10% serum rather than 1% or 5% by day 7 (p-value < .01)

Correlation between Cell Cycle and Different Serum Conditions



10% Serum concentration produced a greater percentage of cells in S phase relative to 1% and 5% serum concentrations (p-value < .05)

Live/Dead Assay Differentiates between Live and Dead Cells using Fluorescence

Treatment	Color	Morphology, Viability
PBS	Green	Elongated, Alive
Ethanol	Red	Round, Dead
PBS + 2 drops Ethanol	Green with 2 small red regions	Elongated, alive with 2 round, dead regions

HDF Cell Proliferation Greater in 10% Serum

- Greater concentration of dividing cells when grown in 10% serum rather than 1% or 5%
- HDF cells grown in 10% serum had a much greater cell density than cells grown in 1% or 5% serum
- The nutrients and growth factors provided by serum promote more efficient growth at concentrations of 10% rather than 1% or 5%

Toxicity of Ethanol to HDF Cells

- Live cells with fully intact membranes stain green because of cytoplasmic dye
- Dead cells, without intact membrane, stain red because of nucleic dye
- Ethanol causes holes within cellular membrane, allowing penetrance of dye in dead cells