Proliferation and Viability of HDF Cells in Vitro

Objectives

- Determine variation in cell proliferation of HDF cells in 1%, 5%, and 10% FBS.
- Obtain a standard curve for HDF cell concentration by MTT Assay.
- Visualize and estimate viability of HDF cells by Live/Dead Assay.

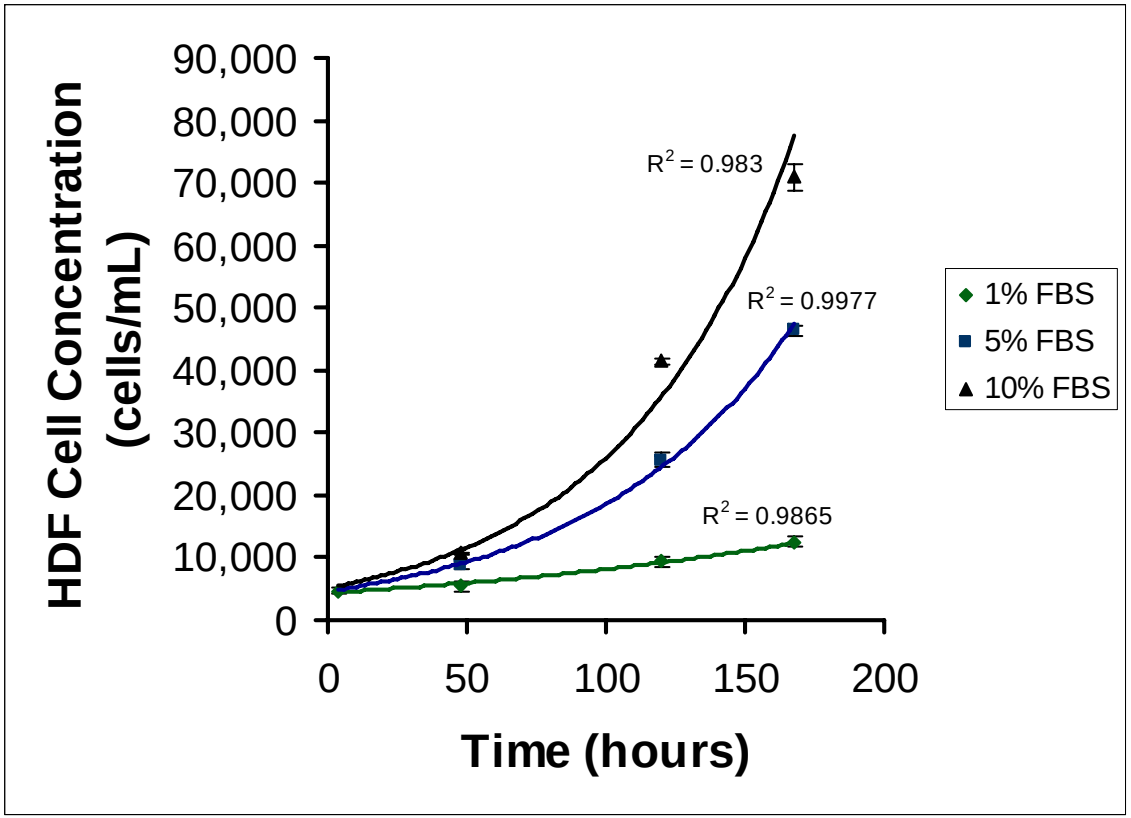
Proliferation Assay Methods

- HDF cells were seeded in 24-well plates and incubated in 1%, 5%, and 10% FBS for 7 days.
  - Cells counts obtained by Coulter Counter
    - Counts made on days 0 (4 hours), 2, 5, and 7 for cells grown in 1% FBS
    - Counts made on days 2, 5, and 7 for cells grown in 5% and 10% FBS

Determination of HDF Cell Viability

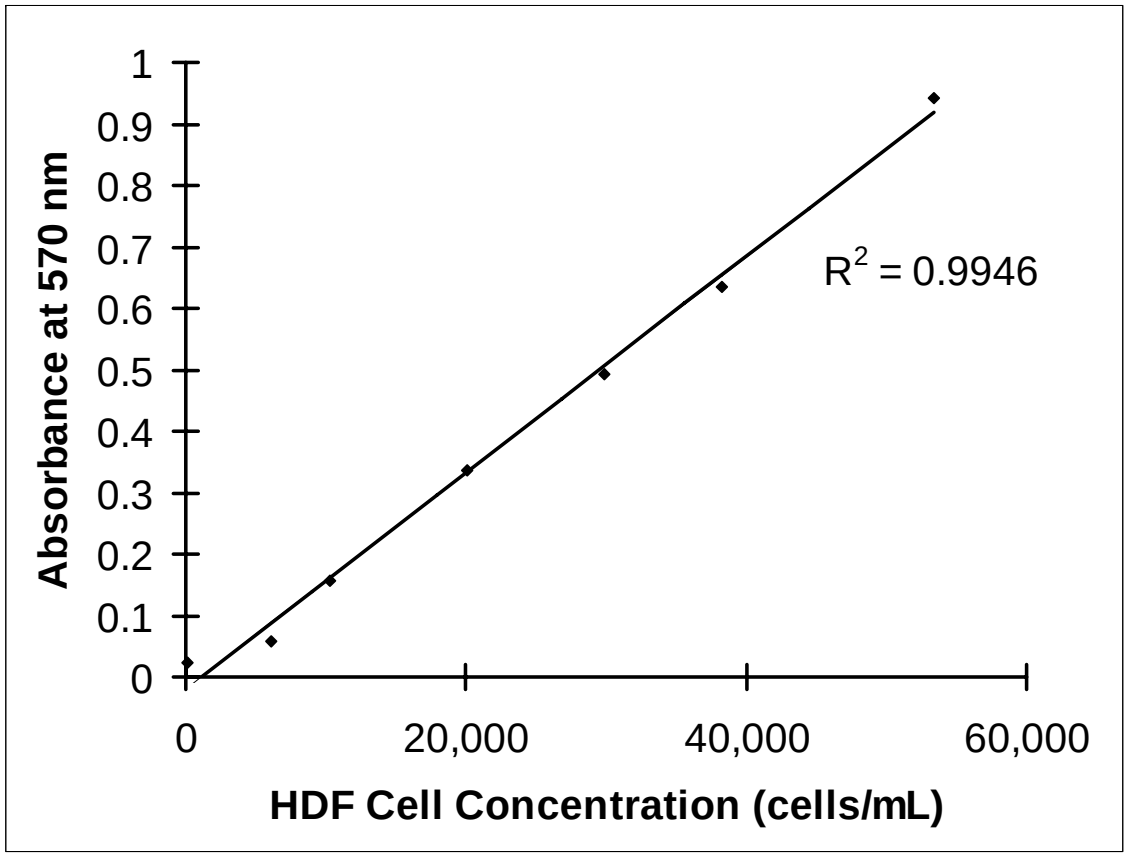
- MTT Assay: Cells seeded with increasing cell concentration and incubated for two days
  - Plate 1: Obtained cell counts by Coulter Counter
  - Plate 2: Cells incubated in MTT dye, which stains metabolically active cells. Absorbance at 570nm was obtained using spectrophotometer.
- Live/Dead Assay: Cells incubated in a 24-well plate for two days
  - Cells treated under three conditions: no ethanol, ethanol, and two drops of ethanol
    - All cell treated with Live/Dead Reagent
  - Cells viewed by fluorescent microscope

HDF Cells Grow Exponentially



*Data from XXX

MTT Assay Standard Curve



*Data from XXX

Live/Dead Fluorescence Assay

Treatment	Color	Morphology	Pattern
PBS, dye	All cells are stained green.	Cells are long and appear attached.	There is an even distribution of green cells.
Ethanol, dye	All cell nuclei are stained red.	Cells are small and round in shape.	There is an even distribution of red stained nuclei.
PBS, 2 drops of ethanol, dye	Some cells are stained green. Some cell nuclei are stained red.	Some cells are long and appear attached while others are small and round in shape.	Even distribution of cells.There is a greater proportion of green cells than red stained nuclei.

Results for Proliferation Assay

- HDF cells exhibited exponential growth over the 7 days.
- On day 7 there were significant differences in mean cell concentrations for HDF cells grown in FBS.
  - 1% and 5% FBS ($p < 0.001$)
  - 5% and 10% FBS ($p < 0.001$)
- As FBS concentrations increased from 1% to 5% to 10%, cell concentrations increased.

Linear Relationship between Cell Concentration and Absorbance

- HDF cell concentration and absorbance at 570 nm are directly related.
- A standard curve can be rapidly obtained and used to make indirect determinations of cell concentration on a large number of samples.
  - Requires fewer cells to run samples for counting than a Coulter Counter

Differential Staining by Live/Dead Reagent

- Living cells appear green under a fluorescent microscope.
- Nuclei of HDF cells lysed by ethanol appear red under a fluorescent microscope.
  - Small amounts of ethanol kill only a portion of the HDF cells.

Comparison of MTT and Live/Dead Assays

- Cell Concentration
  - MTT Assay cell counts made with Coulter Counter are rapid and more accurate.
  - Live/Dead Assay requires visual estimation of live and dead cells, is slower, and can be dependent on human technique.
- Visualization of Viable cells
  - MTT Assay does not permit visualization of live or dead cells.
  - Live/Dead Assay permits visualization of the morphology, distribution, and proportion of live and dead cells.

Summary/Discussion

- Proliferation and viability assays are useful tools for planning experiments.
  - Proliferation Assays provide estimates for the cell concentration after several days based on the seeding concentration.
  - MTT Assay can facilitate comparisons of cell concentrations in experiments which have many samples.
  - Live/Dead Assay can be used to determine a substance's cell toxicity.