In Vitro Investigation

BIOE 342

April 9, 2008

Statement of Purpose

- Measure the effect of various FBS concentrations on the proliferation of HDF cells.
- Compare two methods for determining cell viability.

Cell Proliferation Assay

- Cells seeded at 5,000 cells/well.
  - Samples prepared in triplicate for each test condition.
- Cells were then incubated in media containing different FBS concentrations.
  - 1, 5, and 10% FBS
- Cells were trypsinized and Coulter counted at four predetermined time intervals.
  - 4 hours, 2, 5, and 7 days.

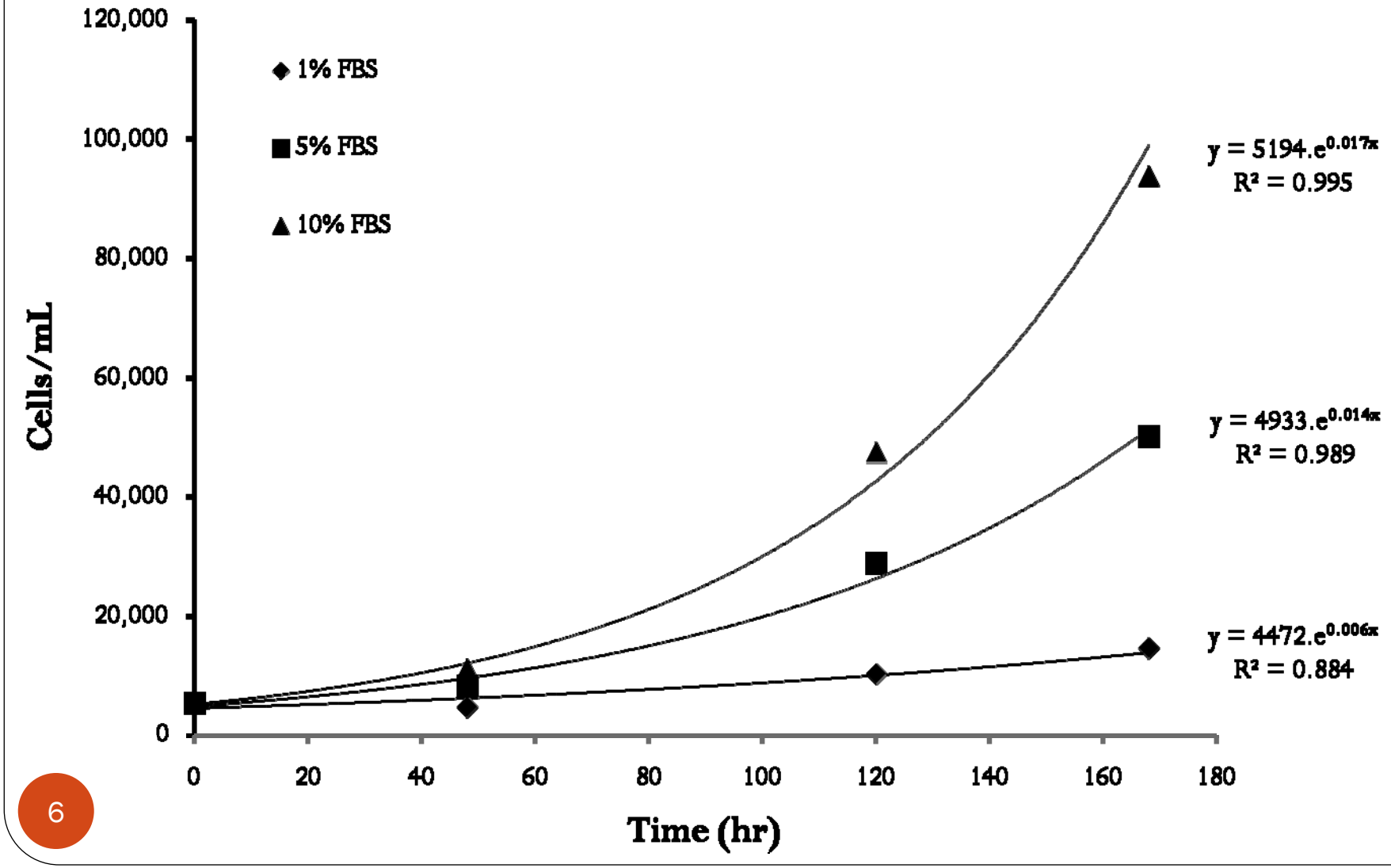
Viability Experiments: MTT Assay

- Stock cells prepared in media (10% FBS) at 50,000 cells/mL.
- Six stock dilutions and control prepared as follows:
  - 2:3; 1:2; 1:3; 1:6; 1:12; and control (100% media).
- After two days of incubation, MTT dye added to each sample and incubated an additional four hours.
- Cells then dissolved in MTT stop solution and transferred to a cuvet.
  - Absorbance read at 570 nm.

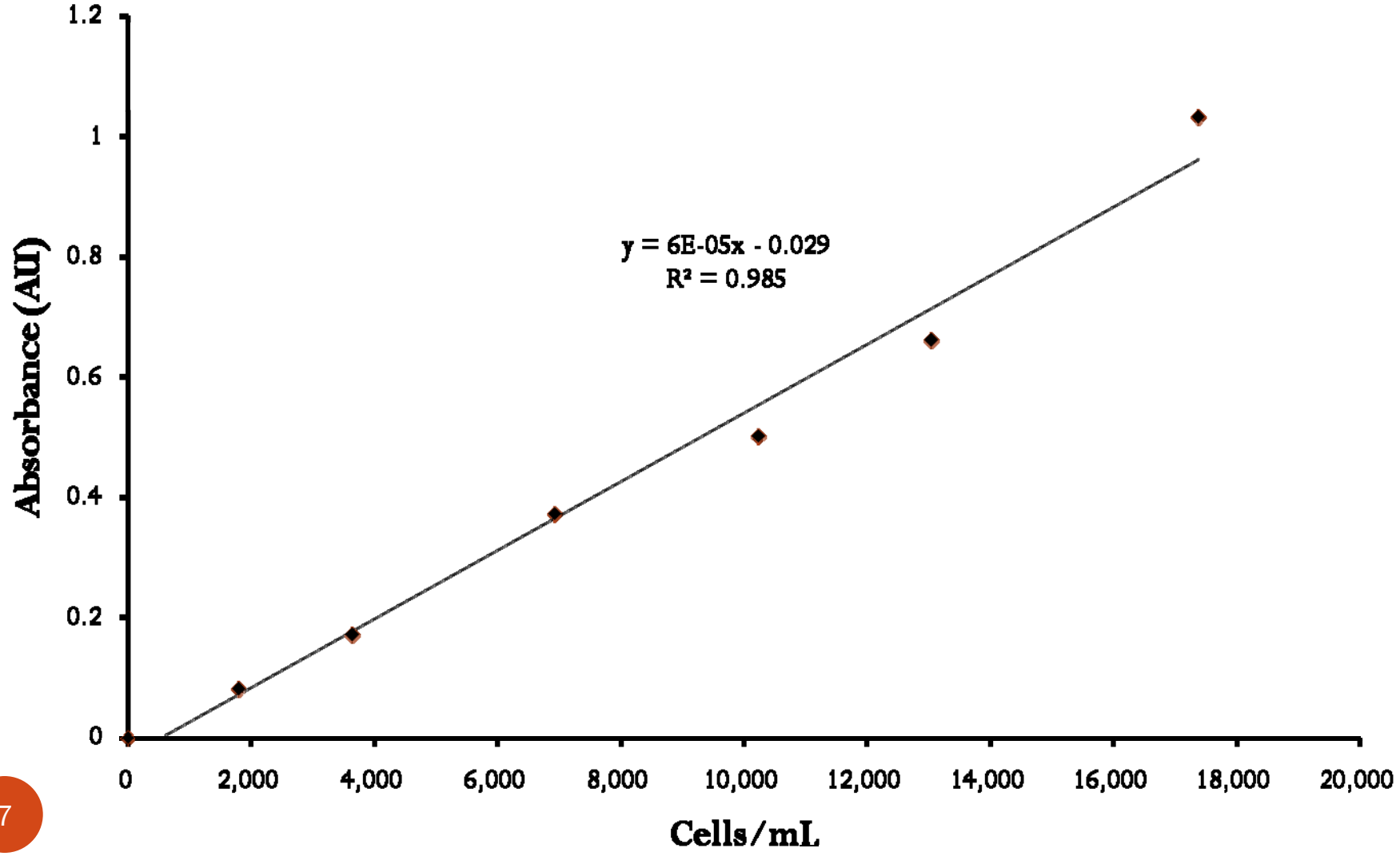
Viablility Experiments: Live/Dead

- Stock cells plated in triplicate for three test conditions.
- After two days of incubation, cells were rinsed (PBS) and treated in three ways:
  - 250 uL PBS and 100 uL Live/Dead stain
  - 250 uL EtOH and 100 uL Live/Dead stain
  - 250 uL PBS, 2 drops EtOH, and 100 uL Live/Dead stain
- After 30 minutes of incubation, cells were viewed using a fluorescent microscope.

Exponential Cell Proliferation is Greatly Effected by FBS Concentration

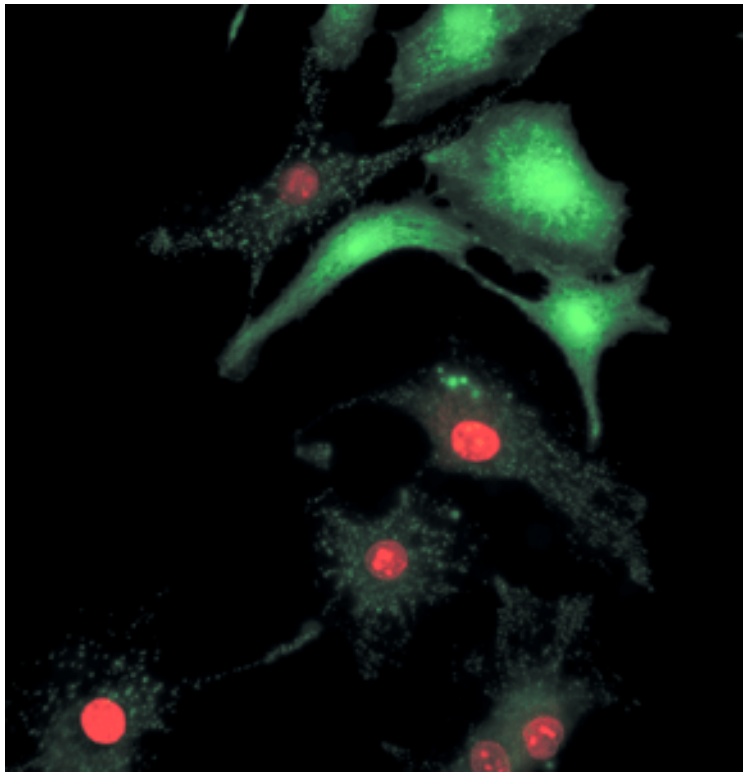


MTT Assay Shows Linear Relationship Between Absorbance and Cell Number



Ethanol Kills Cells

- Live/Dead reagent stains the nuclei of dead cells red.
- Living cells appear green.
- Sample without ethanol contains no dead cells.
- Virtually 100% of cells in sample 2 are dead.
- Sample containing 2 drops of ethanol shows little cell death.



Comparing MTT and Live/Dead Assays

- Live/Dead assay is a more accurate method for determining the number of live cells in a sample.
  - Dead cells are also visible and easily distinguishable from live cells.
- MTT assay relies on the Coulter counter for actual quantification of viable (living cells).
  - Coulter counter also counts dead cells.
- However, MTT assay is a much more time efficient assay as highthroughput screening can be achieved.
  - Live/Dead assay requires manual counting for quantification.

Summary

- Cell proliferation was found to be exponential over time.
  - Each cell type requires a specific amount of FBS in order to optimize cell growth.
- Live/Dead assay is an efficient method to quantify the number of viable cells in a sample.
  - Live and dead cells are easily distinguishable visually.
- MTT assay is a very quick and efficient way to approximate the number of viable cells in a given sample.
  - However, MTT is a more qualitative assay than Live/Dead assay.