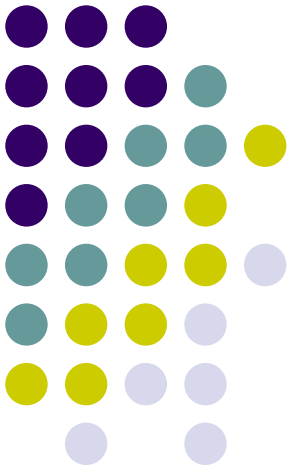
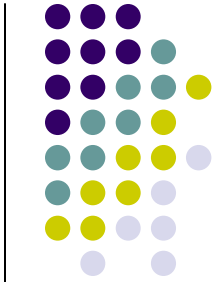
In Vitro Assessment of Human Dermal Fibroblast Viability and Proliferation

BIOE 342

13 Feb 2008

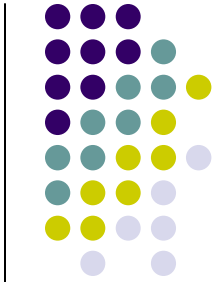


Objectives



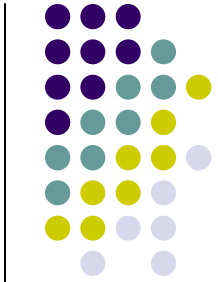
- Determine a relationship between cell concentration and absorbance
  - MTT Viability Test
- Observe fraction of cells in S-phase
  - Anti-PCNA Staining
- Assess cell proliferation over time for different media conditions
  - Cell Proliferation Assay

MTT Viability Test



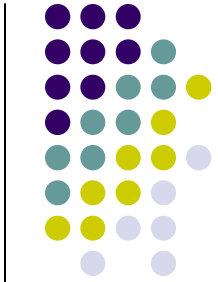
- Seed 6 different cell concentrations 1 mL/well
  - stock (50,000cells/mL) and 1:1.5, 1:2, 1:3, 1:6, 1:12 dilutions
- Incubate cells 2 days
- 2 treatments:
  - Measure cell concentration using Coulter Counter
  - Measure cell absorption using MTT dye and spectrophotometer

Anti-PCNA Staining



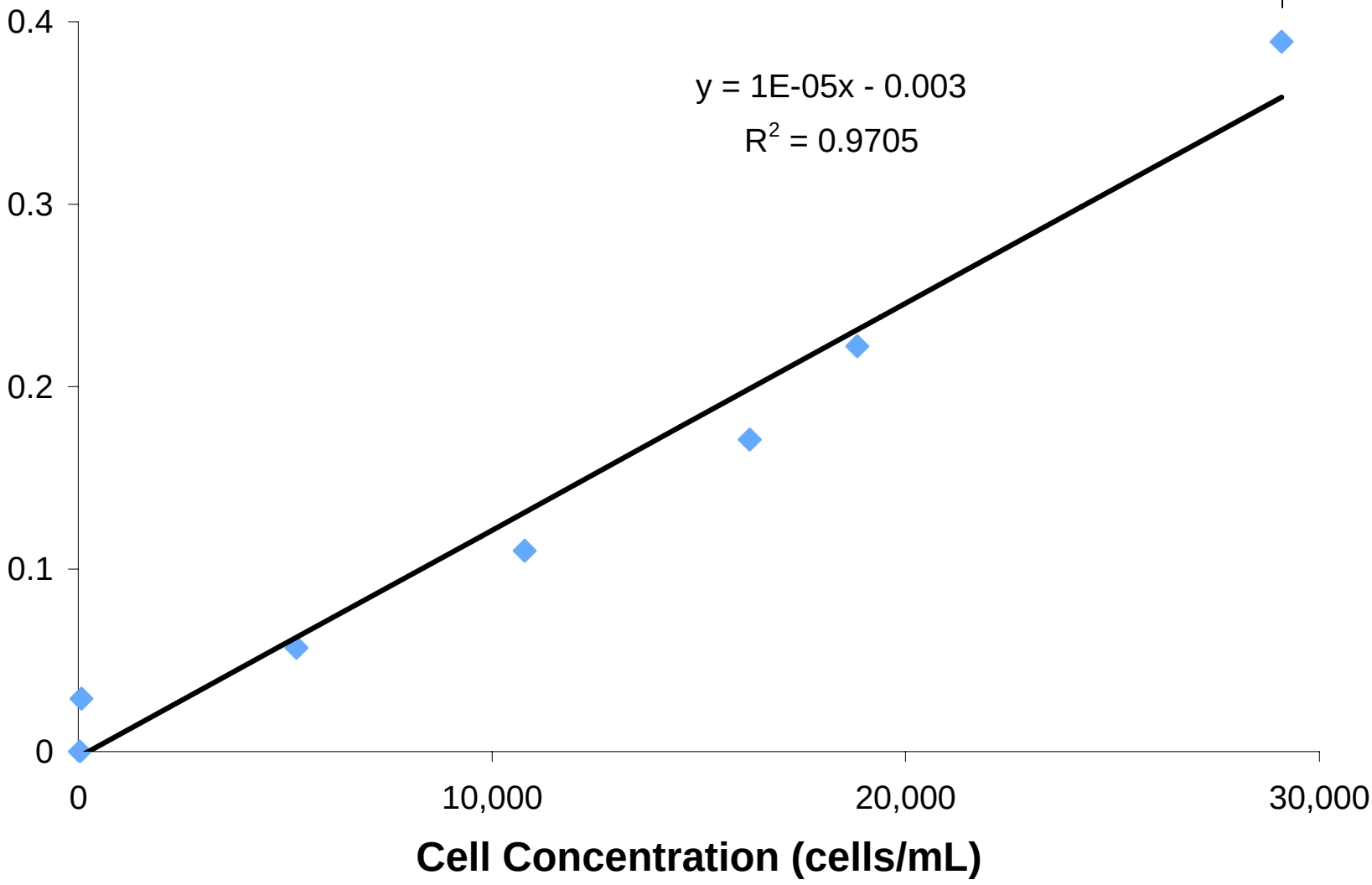
- Seed cells (20,000 cells/well)
  - 3 conditions: 1%, 5%, 10% FBS (fetal bovine serum) in DMEM
- Incubate cells 2 days
- Stain Cells
  - 1º antibody: Mouse IgG to Proliferating Cell Nuclear Antigen (protein with high expression during DNA synthesis)
  - 2º antibody: Anti-Mouse IgG to 1º antibody with Horse Radish Peroxidase (HRP) tag
  - AEC solution: reacts with HRP to stain DNA synthesizing (S-phase) nuclei red
  - Hemotoxylin: stains remaining nuclei not in S-phase blue
- Observe cells under light microscope

Cell Proliferation Assay

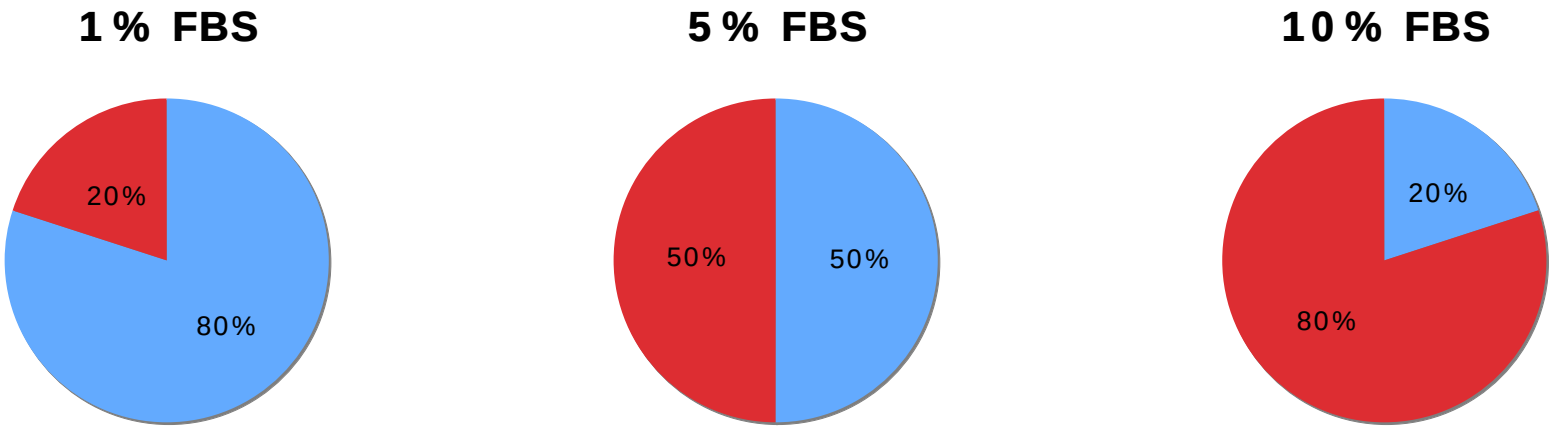


- Seed cells (5,000 cells/well)
  - 3 conditions: 1%, 5%, 10% FBS in DMEM
- Incubate cells
- Count cells using Coulter Counter on days 0, 2, 5, and 7
- Replenish media on days 2 and 5 for cells not counted

Linear Relationship between Absorbance and Cell Concentration

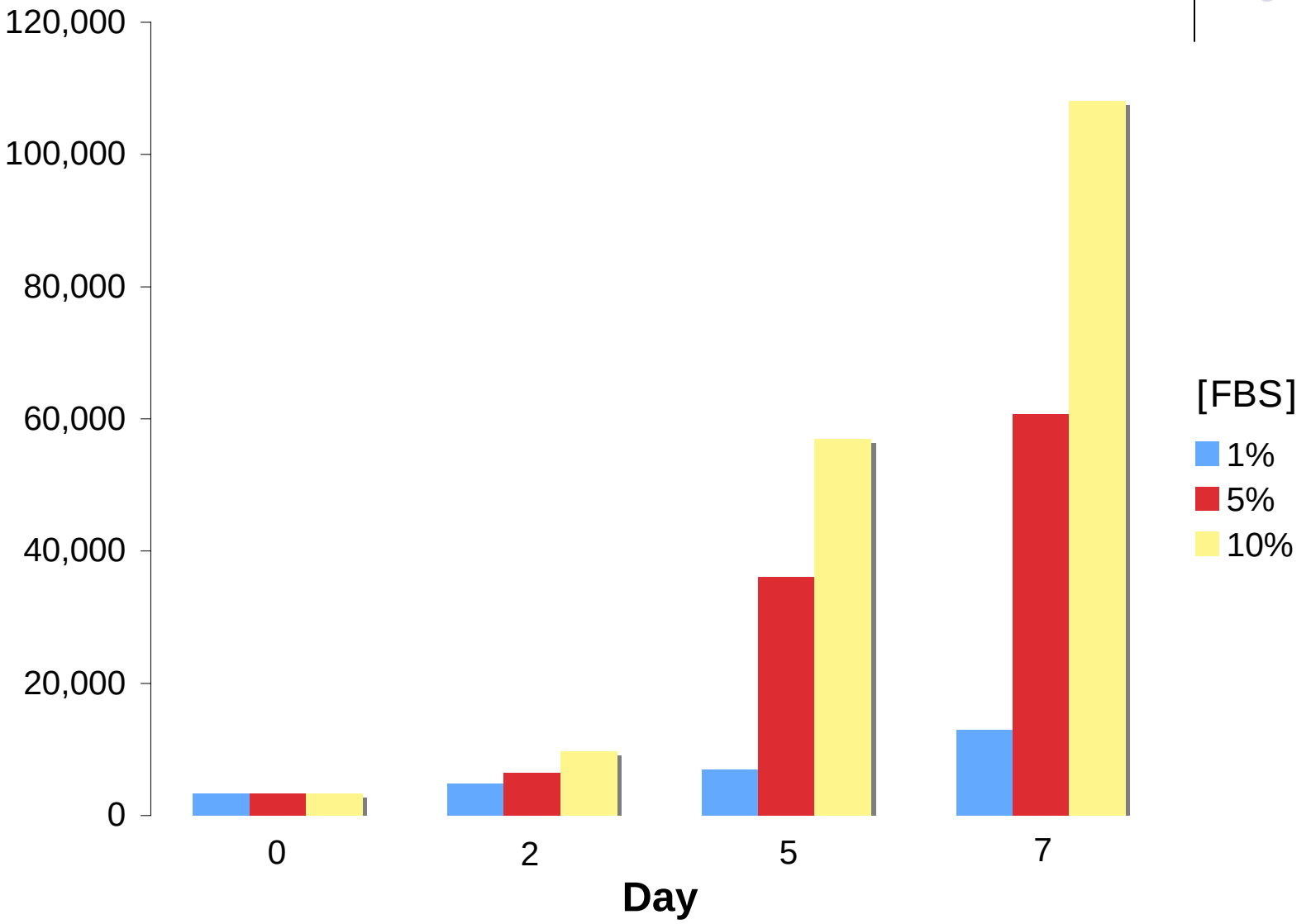


Positive Relationship between FBS Concentration and Proliferation



As FBS concentration increases, so does percent cells in S-phase.

Rate Cell Proliferation Dependent on %FBS



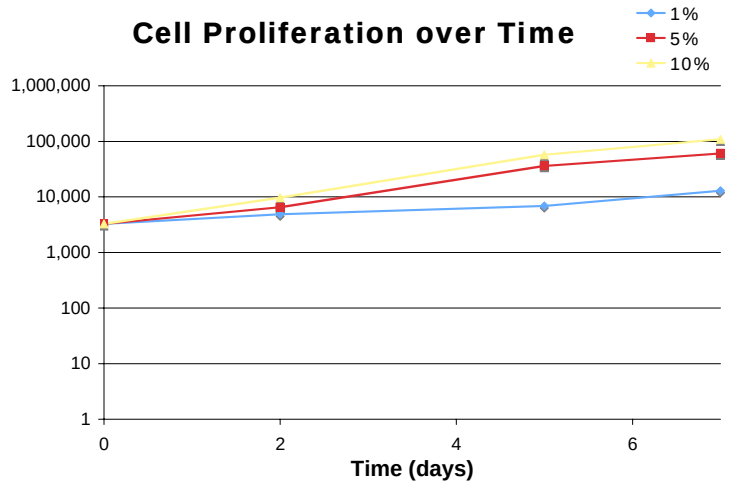
Cell Concentration as a Function of Absorbance

- MTT Viability provides concentration of viable cells in terms of absorbance
  - MTT is a yellow dye that is reduced by live cells to a purple formazan product insoluble in aqueous solutions[1]
- Absorbance can be linearly related to cell number by counting cells on Coulter Counter

[1]Freshney, R. I. *Culture of Animal Cells*.

Proliferation of HDF Cells

- Anti-PCNA staining and the Cell Proliferation assay related cell proliferation to FBS concentration in media
- Increased FBS concentration causes increase in cell proliferation
  - Serum contains growth factors that promote cell proliferation[1]
- Cell Proliferation Assay shows exponential growth of HDF cells



Anti-PCNA Staining vs. Cell Proliferation Assay

	Anti-PCNA Staining	Proliferation Assay
Media Conditions	+	+
Quantitative	-	+
Time (Days)	2	7