Environmental Effects on HDF Proliferation

BIOE 342 – Section 2

Objectives

- Observe the effect of Fetal Bovine Serum (FBS) on the proliferation of Human Dermal Fibroblasts (HDF)
 - Compare the growth rates/stages of HDF in different FBS concentrations
- Find a linear relationship between cell concentration and absorbance of metabolic dye (MTT)

Using Absorbance from MTT Assay to Calculate Cell Concentration

- Seed HDF cells: stock (50,000 cells/ml) and dilutions of 1:1.5, 2, 3, 6 and 12
- MTT dye added (2 hour incubation for metabolism to occur)
- Harvest cells for counting
 Coulter Counter (Z1)
- Record absorbance at 570 nm using spectrophotometer (Genesys 10UV)

Anti-PCNA Stains S-phase Nuclei Red

- Seed uniform concentration of HDF
 In DMEM with 1%, 5% or 10% FBS
 2 day incubation
- Dye cells using Anti-PCNA, Horseradish Peroxidase and AEC solution (specific to S-phase nuclei) and hematoxylin (nonspecific dye)
- Observe cells using light microscope

Determine Effect of FBS Concentration on Growth Rate

- Seeded cells (5,000 cells/ml) in DMEM, 1% FBS initially
 - Harvest times: 4 hours and 2, 5, and 7 days after seeding
- At each time point:
 - □ Fed cells for successive time points: DMEM with 1%, 5% or 10% FBS
 - Harvested current cells and counted using Coulter Counter (Z1)

MTT Absorbance Increases Linearly with Cell Concentration



Higher FBS Yields Greater Number of Cells in S-phase

Condition	% Red	Interpretation
(%FBS)	Nuclei	
1 %	~1%	Low FBS concentration → Very few cells are replicating
5 %	5%	An increase in number of replicating cells proportional to FBS increase
10 %	10%	Maximum FBS concentration tested yields maximum S- phase cells observed 7

Greater Exponential Growth for HDF Cells in Higher FBS Concentrations



Greater Exponential Growth for HDF Cells in Higher FBS Concentrations

- Significantly greater growth occurred for cells in higher percent FBS (Anova, p< 3.2E-5)
- Cell doubling times
 - □1% 5.8 days
 - □5% 1.9 days
 - □10% 1.6 days

Cell Proliferation Study and Anti-PCNA Assay Agree

- Doubling time decreases as % cells in Sphase increases
- Final cell concentration increases linearly with % cells in S-phase (R² = 0.99)
- Higher % FBS results in more cell replication, and therefore greater growth

Conclusions

FBS concentration in DMEM has direct effect on cell proliferation

□Higher FBS concentration:

- Greater cell concentration after 7 days (Anova, p< 3.2E-5)
- Anti-PCNA: greater number S-phase cells

Doubling time shorter

 Absorbance from MTT assay has linear relationship to cell concentration (R²=0.97)