Human Dermal Fibroblast Cell Proliferation and Viability in Vitro

YYY

BIOE 342

May 27, 2009

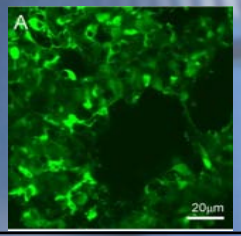
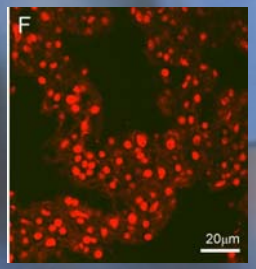
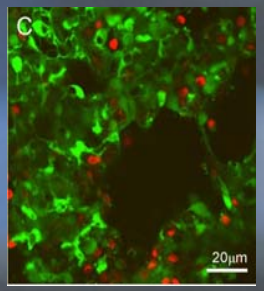
Experimental Objectives

- To assess qualitatively Human Dermal Fibroblast (HDF) viability with fluorescence imaging
  - Live/Dead Fluorescence Assay
- To measure quantitatively the effects of serum concentration on HDF growth and proliferation
  - Anti-PCNA Staining Assay
  - Cell Proliferation Assay

Live/Dead Fluorescence Assay

- Preparation: HDF cells seeded in 24-well TC treated plates
  - Incubated 2 days
  - Dyed with solution of Calcein AM and EthD-1
- Conditions (3 wells each):
  - A. Control
  - B. 1 mL Ethanol
  - C. 2 drops Ethanol
- Equipment: Fluorescent microscope

Dyed Cells Fluoresce Green When Alive and Red When Dead

Condition	Example[1]	Observations
Dye alone		All cells are stained green. Cells are elongated and attached.
Dye, 1 mL Ethanol		All cell nuclei are stained red. Cells are mostly rounded.
Dye, 2 drops Ethanol		Some green cells interspersed with red nuclei. Some elongated and rounded cells. There are more green cells than red stained cells.

HDF Cells Are Permeable to Ethanol

- HDF membranes allow the diffusion of ethanol into the cell
- Ethanol is disruptive to HDF cell environment and results in visible cell necrosis
- Small amounts of ethanol kill only a portion of the HDF cells
- We may use live/dead assays to asses the cytoxicity of any other substance

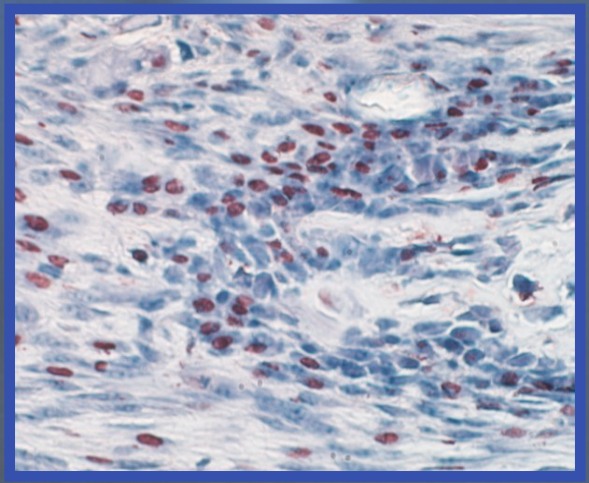
Anti-PCNA Staining Assay

- Preparation: Cells seeded at ~20,000 cells/mL in 24-well TC treated plates
  - Incubated 2 days
  - Fixed in Formalin
  - Primary Antibody (Ab): Anti-PCNA Mouse IgG
  - Secondary Ab: Anti-mouse IgG tagged with HRP
- Conditions (1 well each):
  - Dulbecco's Modified Eagle Medium (DMEM) with 1,5 and 10% Fetal Bovine Serum (FBS)
  - In addition, 3 controls seeded with 10% FBS DMEM
- Equipment: Light microscope

Fraction of HDF in S Increases with %FBS

- HDF in S phase are preparing for division
  - PCNA production at a max in S phase
- PCNA tagged by primary and secondary Ab
  - HDF cells in S phase stain red[2]
- Percentage of HDF in S phase increases 85% from 1 to 5 % FBS, and 74% from 1 to 10% FBS

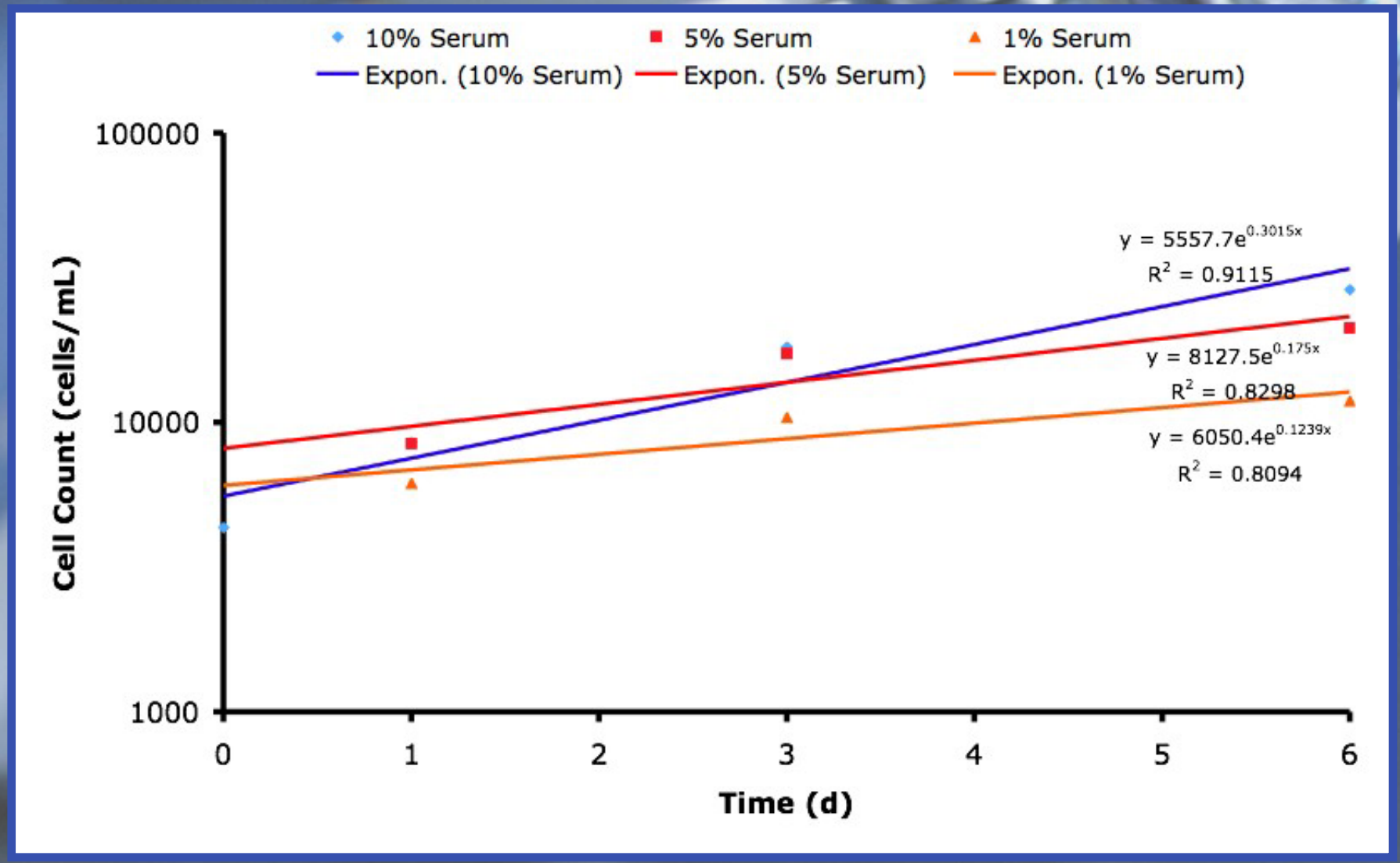
Condition	Confluence	Cell Staining Observations
1% FBS	60%	50% blue, 50% red
5% FBS	60-80%	7.2% blue, 92.8% red, many double nuclei visible
10% FBS	80%	13% blue, 87% red, double nuclei visible



HDF Proliferation Assay

- Preparation: Cells seeded at ~5000 cells/mL in 24-well TC treated plates
  - Media replenished on day 1
- Conditions (Data obtained at 0,1,3 and 6 days):
  - DMEM with 1,5 and 10% FBS
- Equipment:
  - Light microscope
  - Coulter Counter

HDF Growth Curve is Exponential and Related to %FBS



10% FBS Yields Higher Cell Count Than 1% and 5%

- As % FBS increases from 1 -10%, [cell] increases exponentially (R^2>0.8)
- Day 3 cell count means among 1, 5 and 10% FBS are statistically different as determined by ANOVA with Tukey's HSD (p=0.0447 <0.05)
- Doubling times decrease with increasing %FBS

Coulter Counter Data (Day 3)

1% FBS	5% FBS	10% FBS
682	935	648
350	871	1075
433	785	1015

Serum (%)	Time to double (d)
10%	2.3
5%	3.96
1%	5.59

Proliferation and Anti-PCNA Results Show Same Trend

- Both assays illustrate HDF cells' propensity towards FBS in media
- Increasing serum concentration from 1 to 10% results in:
  - Increased final (day 6) cell concentration by 74%
  - Decreased doubling time by 41%
  - Larger proportion of cells in S phase (More viable proliferating cells) by 85%
- FBS nourishes cells with nutrients and growth factors
  - Increasing its concentration in media is strongly advised at least up to 10%

Key Results

- Cytoxicity may be assessed using fluorescence imaging as in the Live/Dead assay
  - Ethanol decreases cell viability by quickly disrupting the cell environment
  - Qualitative data: morphology, spread, distribution
  - Visualization is subject to human error
- Proliferation assays provide quantitative data from which to derive the exponential growth curve relationship as well as doubling times
  - Varies with %FBS
- Anti-PCNA assays show the fraction of cells that are likely proliferating
  - Log phase of the growth curve
  - Varies with %FBS

Work Cited

- 1
  - http://respiratory-research.com/content/figures/1465-9921-6-40-1-l.jpg
- 2
  - http://www.cor.uams.edu/images/youngmouse_do%20pcna40x.jpg