HDF Survival and Function

June 2, 2008

Objective

- To assess the impact of toxic material on living HDF cells
- To observe proliferation and media conditions using antibody specific staining procedures
- To quantitatively assess the effects of serum on HDF cell growth and replication

Measuring Live & Dead Cells

- HDF cells placed on TC-treated plates and incubated for 30 min. after treatment
  - Three conditions: live, dead, live/dead mixture
- Cells treated with live/dead reagent
- Ethanol added to dead and mixture wells
- Observations made using a fluorescent microscope

Anti-PCNA Staining Methods

- Cells seeded at 20,000 cells/mL
  - Three wells with 1%, 5%, and 10% FBS respectively and three control wells
- Incubated for two days
- Antibodies and Hematoxylin stain solution added
- Stained cells observed with a light microscope
  - Red : cells in S-phase
  - Blue: all other cells

Measuring Cell Proliferation

- Cells seeded at 5,000 cells/mL
  - Day 0 - all 1% serum
  - Day 1, 3, 7 - 3 wells of 1%, 5%, 10 % serum each
- After appropriate incubations, cells trypsinized and counted using Coulter Counter

Ethanol Effectively Kills HDF Cells

Condition	Ethanol	Observations	Viability
A	0	mostly green	30% dead
B	250 µL	all red	100% dead
C	2 drops	mixture red/green	40-50% dead

- Fluorescent microscope used:
  - Green - live cells
  - Red - dead cells
- Ethanol kills HDF cells as seen in conditions B and C

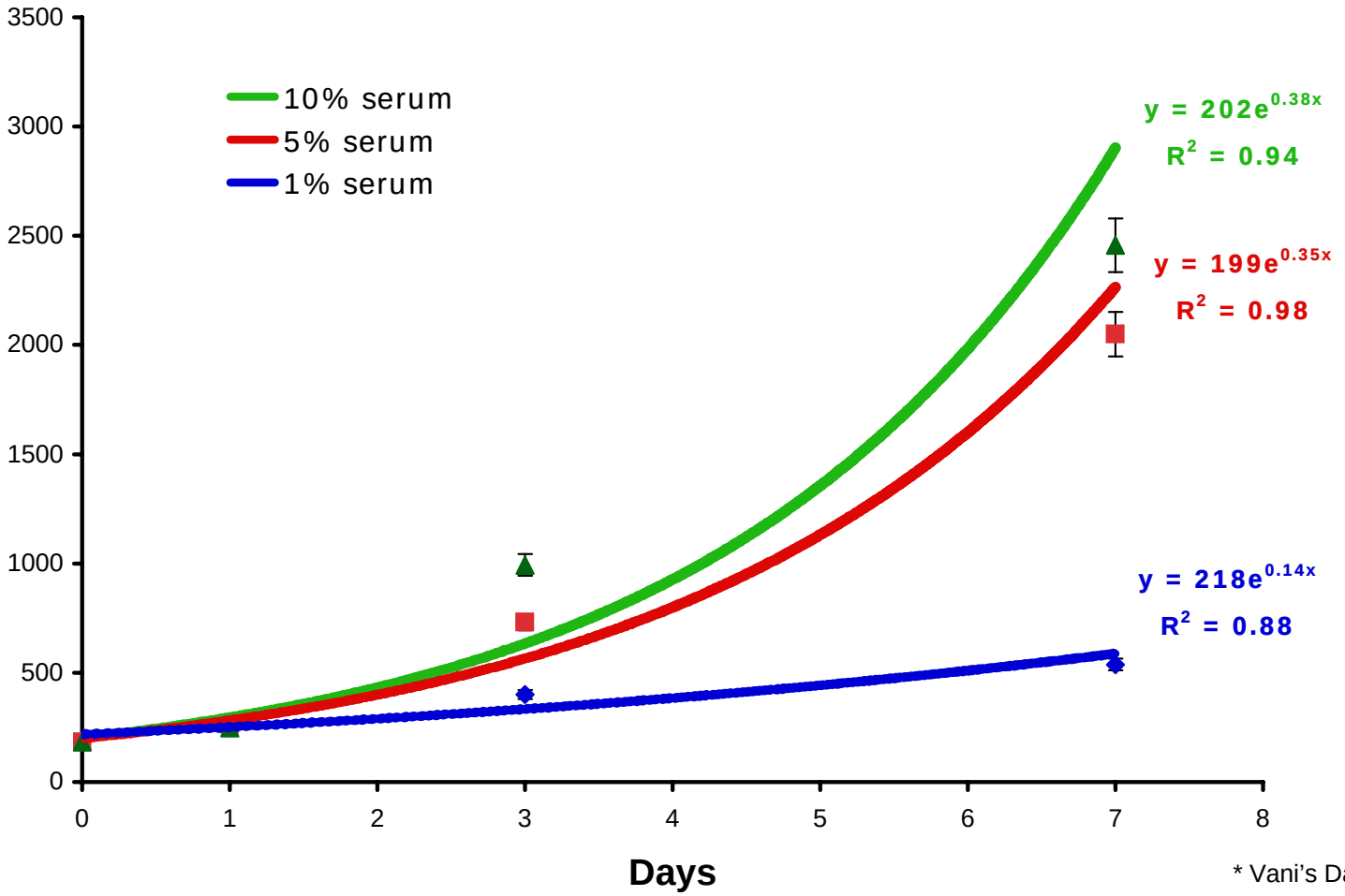
Increased Serum Yields Increased S-phase Nuclei

Condition	% Serum	Confluency	Color
A	1%	50-60%	50% red
B	5%	60-70%	60% red
C	10%	60-70%	60% red
Control	10%	--	100% blue

- Controls not given both antigens
  - Primary and secondary antibody must be present for proper nuclei staining
- Increased serum percentage increased number of cells approaching cell division

* Jimmy's Data

Cell Number Increases with Increasing Serum Concentration



* Vani's Data

Increased Serum Percentage Yields Increased Number of Cells

- All three conditions displayed exponential growth as time increased
- The 5% and 10% serum conditions showed a significantly steeper slope than the 1%
  - 10% serum yielded greatest number of cells
- R^2 values for all three curves indicate accurate treadlines

Anti-PCNA and Proliferation Correlations

- Anti-PCNA found a correlation between serum percentage and the number of cells approaching replication
- Cell Proliferation observed that an increase in serum percentage increases cell growth
- 10% serum had the greatest number of cells approaching replication and therefore the greatest number of cells

Conclusion

- Ethanol effectively kills cells
- Increased serum percentages:
  - yields a greater number of cells in S-phase
  - increases number of cells proliferating