FIBROBLAST VIABILITY AND GROWTH IN VARYING MEDIA CONCENTRATIONS

Student Name
13 February 2008

DRAFT

Objectives

- To qualitatively and quantitatively determine the viability of human dermal fibroblasts (HDF) in varying concentrations of fetal bovine serum (FBS) in Dulbecco's Modified Eagle Medium (DMEM) growth media
  - Anti-PCNA Staining
  - Cell Proliferation Assay
- To stain and visually assess between living and dead cells
  - Live/Dead Fluorescence Assay

Anti-PCNA Staining Illuminates Cells Undergoing DNA Replication

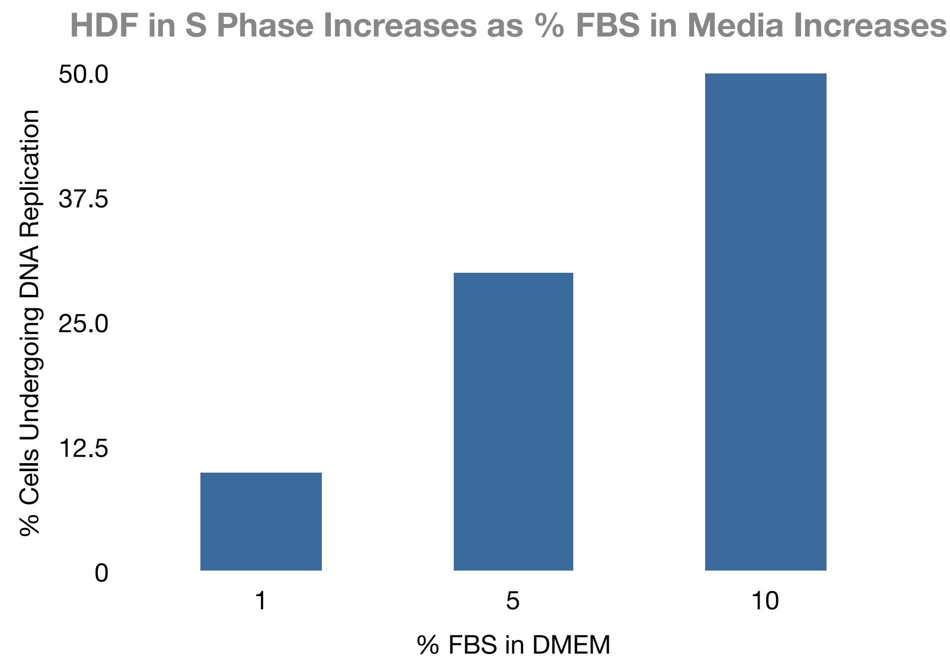
- 20,000 HDF were seeded into three wells, containing DMEM with 1% antibiotic and 1%, 5%, or 10% FBS. Three control wells with 10% FBS also seeded

- All six wells incubated for two days, prepared by formalin and cleansing agents, followd by Anti-PCNA primary antibodies then Anti-mouse IgG secondary antibodies

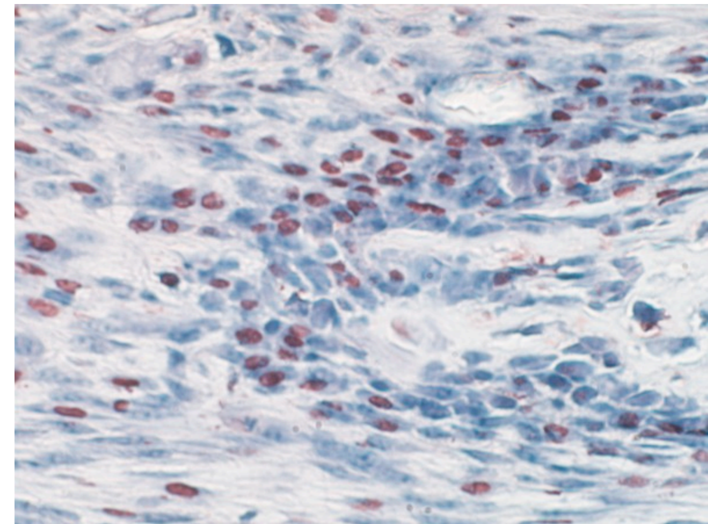
- AEC added, causing a red color to be observed in S-phase cell nuclei created by action of the secondary antibody

- Hemotoxylin added to stain all cells blue

Higher Concentration of FBS Increases Percent of HDF In S Phase



- Red nuclei indicate active participation in S Phase of cell cycle. All other cells and cytoplasm of DNA-replicating cells are stained blue.

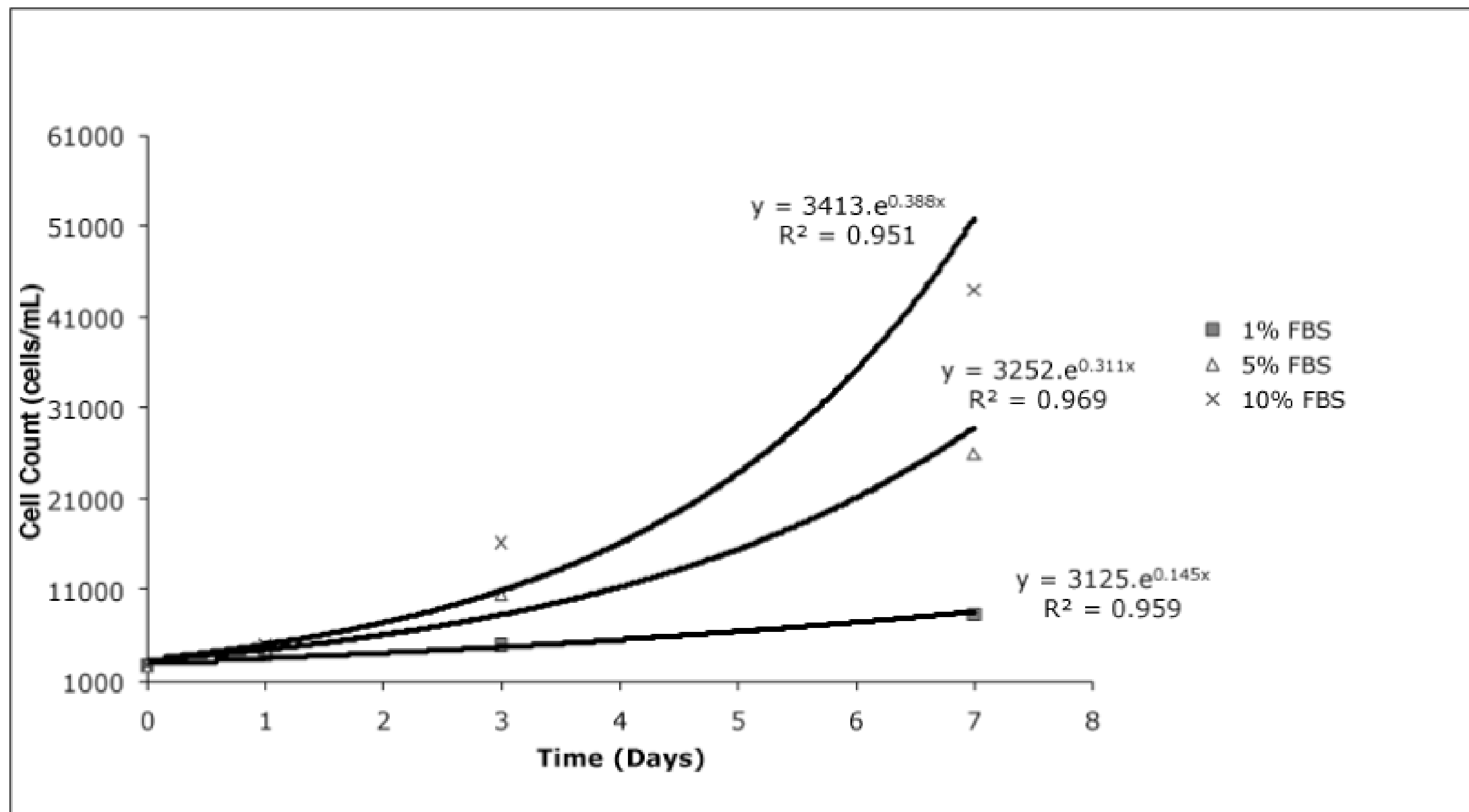


http://www.cor.uams.edu/images/youngmouse_do%20pcna40x.jpg

Cell Proliferation Assay Quantitatively Determines Effects of FBS Concentration

- 5,000 HDF were seeded into 33 wells containing DMEM with 1% antibiotic and 1%, 5%, or 10% FBS
- Cells were incubated for 4 hours, or 1, 3, or 7 days
- Media was replaced every other day for the 3 and 7 day assays
- Cells counted with a Coulter counter

HDF Exhibits Exponential Growth in Each Media Condition

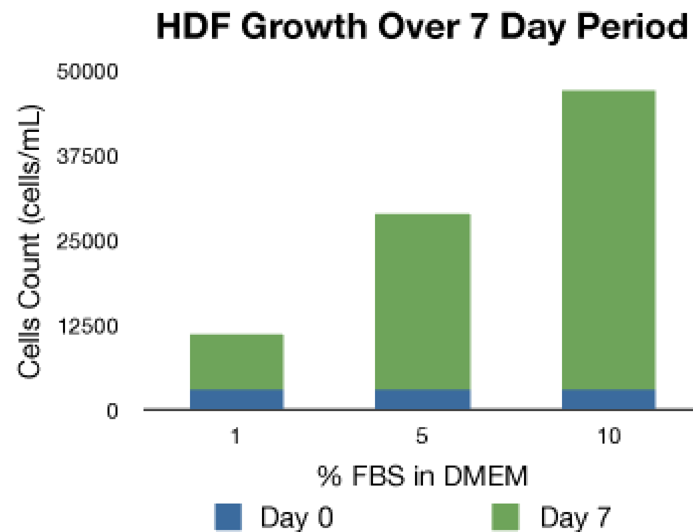


3 measurements taken and averaged for each data point on graph; variation for each set of points averaged is less than 50%, error bars to come for final draft

HDF Doubling Time Statistically Significant Between Each Concentration of FBS

- Exponential growth with diminishing returns can be seen as FBS concentration increases

- Statistically significant variance between all FBS concentrations ($p = 0.05$)



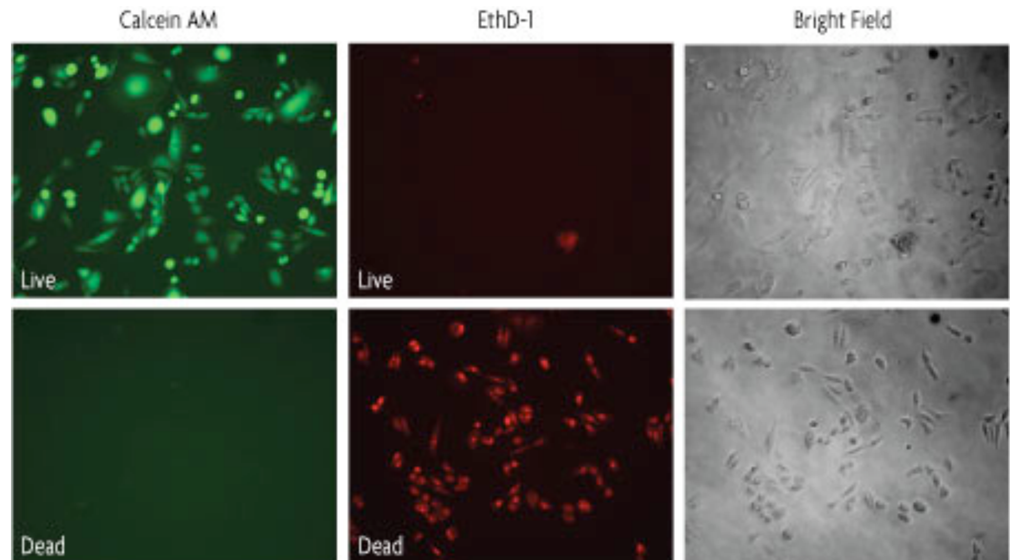
% FBS in DMEM	Doubling Time (Days)
1	4.8
5	2.2
10	1.8

Live/Dead Fluorescent Assay Visually Differentiates Living and Dead Cells

- HDF cells seeded into nine wells and incubated for two days to ensure complete attachment of living cells
- All cells exposed to 100 µL live/dead dye, and either 0 µl, 25 µl, or 250 µL ethanol
- Calcein AM dye targets and stains living cells, Ethidium Homodimer (EthD-1) targets and stains dead cell nuclei
- Cells incubated for 30 minutes at room temperature then observed under fluorescent and light microscopes

Living Cells Stained Green, Dead Cells Stained Red; Only Visible Under Fluorescent Microscope

- Live cells are seen in a vivid green stain; dead cells' nuclei are seen on a different imaging plane in a muted red under a fluorescent microscope.
- Under a bright field microscope, live and dead cells are not differentiable.



- http://www.activemotif.com/catalog/fluorescent_detection/toxcount

- Cells under no ethanol all living (green), cells under 250 μL all dead (red), cells with 25 μL mixed (not pictured)

Concentration of FBS in Media Directly Relates to HDF Growth; Staining Can Determine Between Live/Dead and S-Phase/G-Phase Cells

- Increased concentration of FBS in DMEM leads to exponential increase of HDF growth, caused by increased percentages of cells actively dividing at a given time, and not by increased division speed

- A staining technique allows S-Phase cells versus cells in other stages of the cell cycle to be observed under a bright field microscope.

- A separate staining technique using two types of dye allows visual determination between living and dead cells under a fluorescent microscope