Human Dermal Fibroblast Cells are Dependent on their Liquid Environment for Survival and Growth *in vitro*

BIOE 342

Spring 2008

Response of Human Dermal Fibroblast Cells

- Effect of ethanol on Human Dermal Fibroblast (HDF) cells
  - To determine cytotoxicity of ethanol, a common cleaning chemical
- Effect of serum concentration on proliferation
  - To optimize proliferation for experiments requiring a large number of cells

Live-Dead Assay

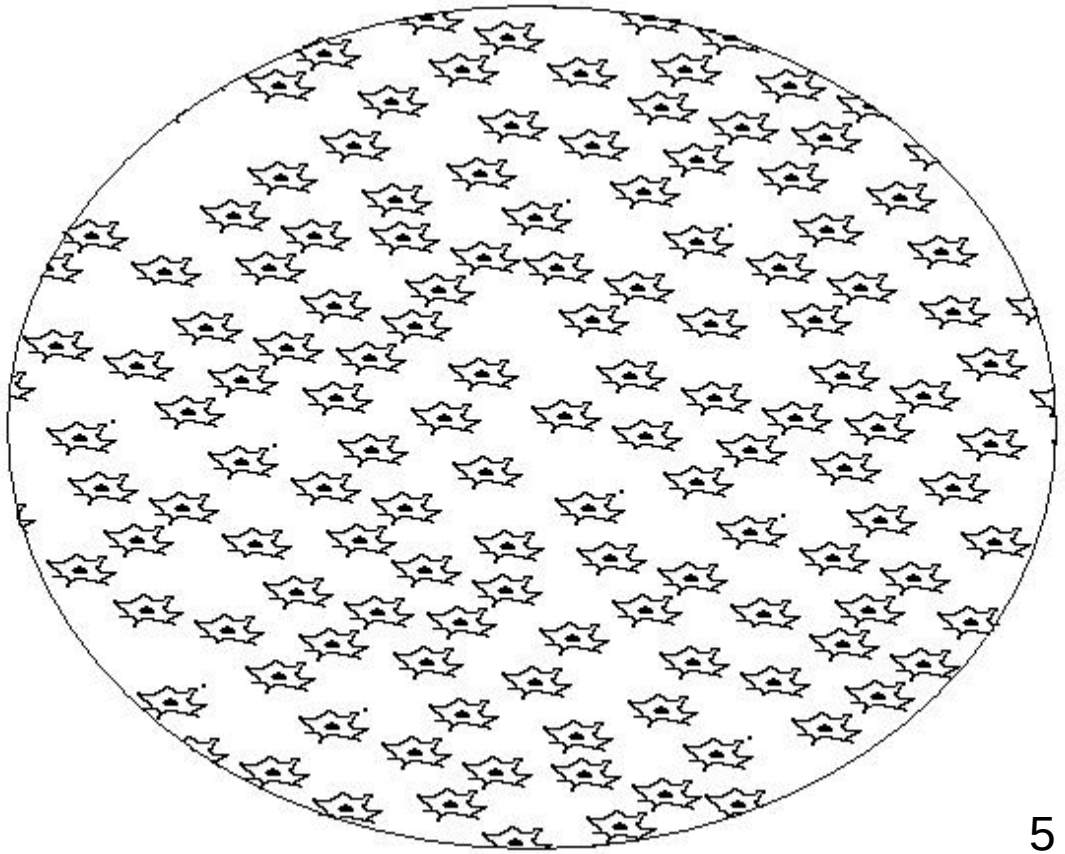
- Cells incubated with Live/Dead stains for ½ hour in:
  - DMEM with 10% FBS
  - DMEM with 10% FBS and drops of 100% ethanol (EtOH)
  - 100% EtOH
- Live-Dead reagents stained live cells green and dead cell nuclei red
- Live/dead cell patterns observed with Nikon fluorescent microscope

Cell Proliferation Assays

- Anti-PCNA staining
  - Cells incubated in DMEM with 1%,5%,or 10% FBS for 3 days
  - PCNA stain
    - All cell nuclei stained blue
    - Cell nuclei in S-phase stained red
  - Ratio of dividing vs. not-dividing cells observed under light microscope
- Quantitative Cell Proliferation Assay
  - Cells incubated in DMEM with 1%,5%,or 10% FBS for 7 days
  - Media changed and cells counted with a Coulter Counter on days 0,2,5,7

Ethanol Does Not Appear to Kill Cells

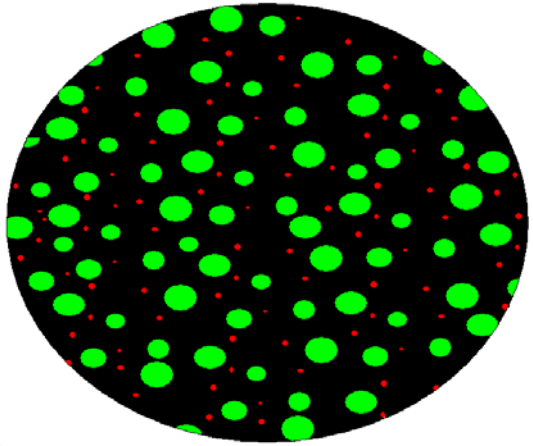
- All conditions appeared to have healthy cells under light microscope.

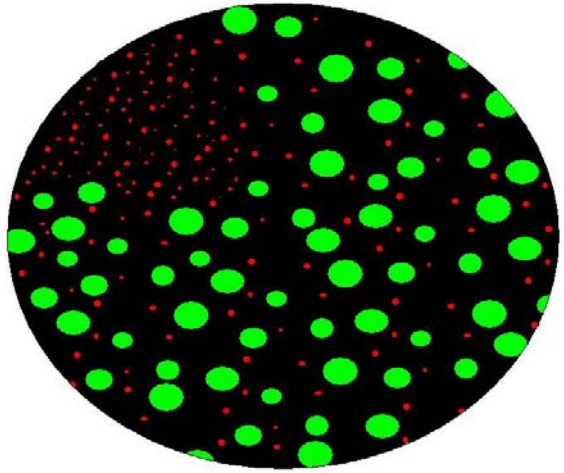


Ethanol Does Kill Cells

- The fluorescent microscope reveals
  - Some dead cells in 10% media – stress of washing
  - Drops of EtOH kill cells where they fall
  - 100% EtOH kills all cells

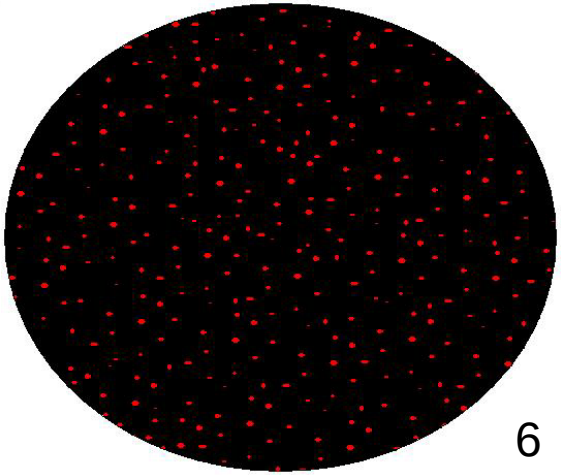
DMEM +10% FBS





DMEM +10% FBS
+EtOH drops

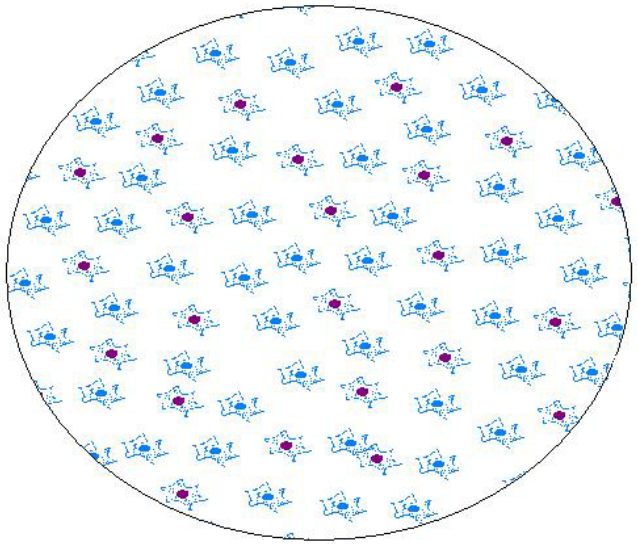
100% EtOH



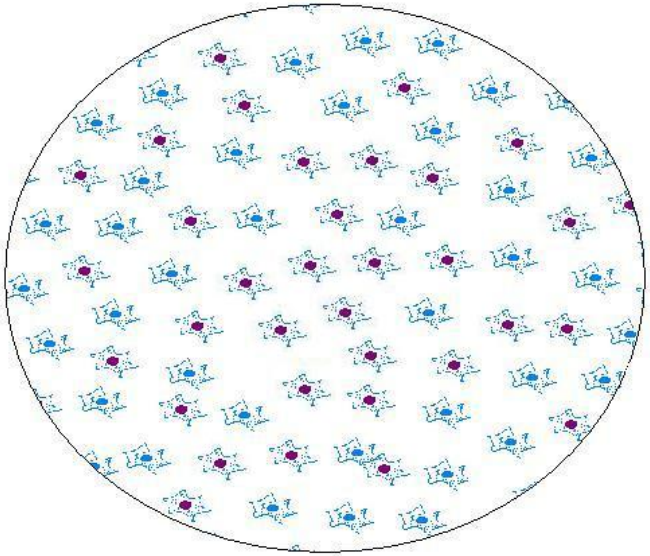
Anti-PCNA Staining Reveals More Cells in S-phase with Increasing Serum Concentration

- After 2 days, more cells in 10% serum were in S-phase (committed to dividing) than cells in 5% serum, and more in 5% than in 1%

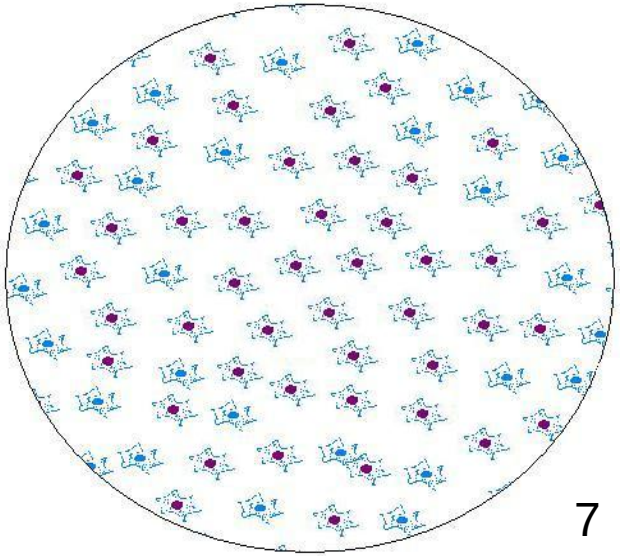
DMEM with 1% FBS
~1:2 dividing:not-dividing



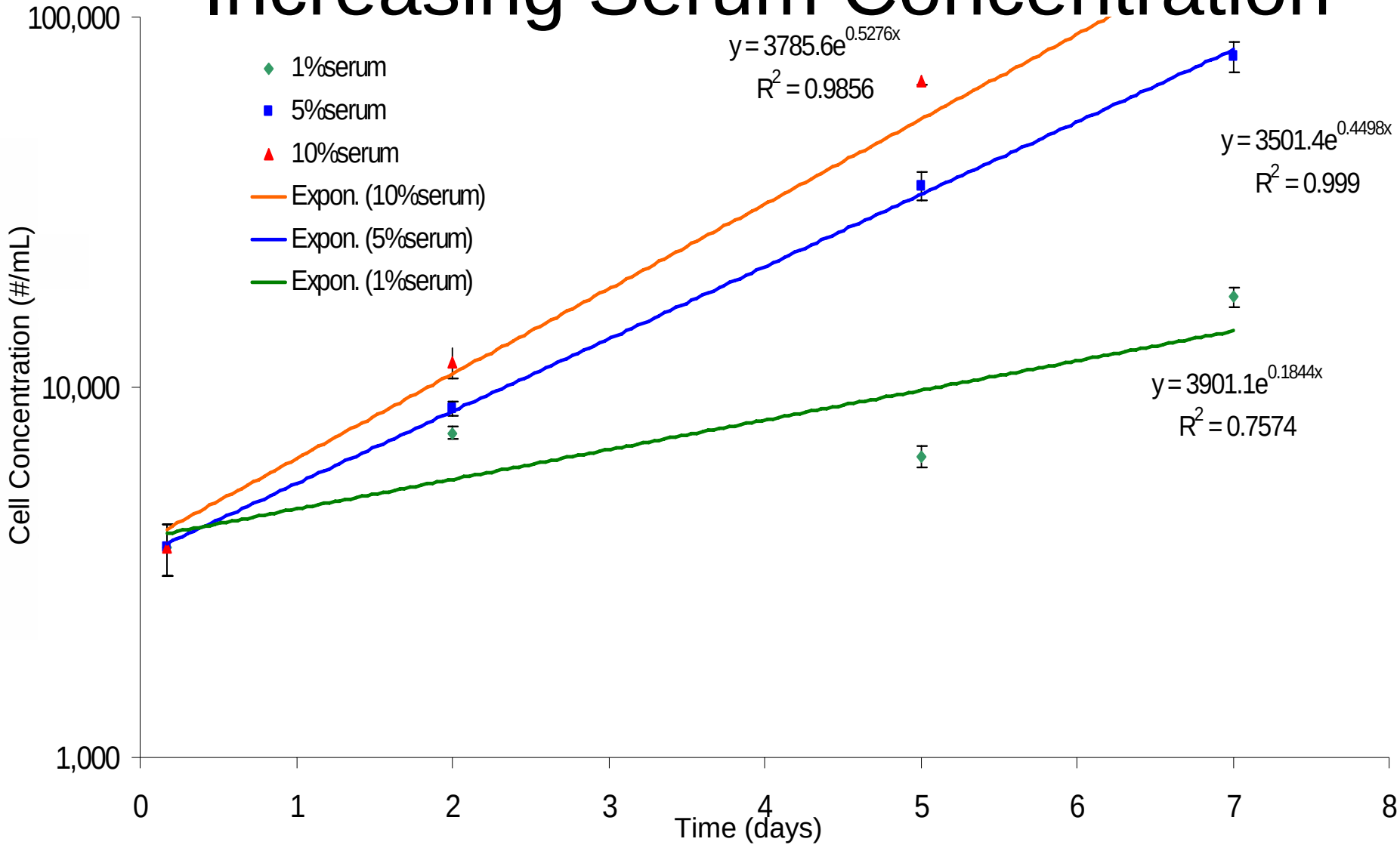
DMEM with 5% FBS
~ 1:1 dividing:not-dividing



DMEM with 10% FBS
~ 2:1 dividing:not-dividing



Growth Rate Increases With Increasing Serum Concentration



Serum Concentration Has an Effect on Cell Growth Rate

- From 1 to 10% serum in DMEM, an increase in serum concentration causes a corresponding increase in growth rate.
- A one factor ANOVA reveals that the difference in growth rates among 1%,5%, and 10% serum is statistically significant ($F>F_{crit}$, $p=4.36e-7$).

Serum Improves Growth Rate by Stimulating Mitosis

- Anti-PCNA stain
  - More cells are in S-phase at higher serum concentrations.
- Quantitative proliferation assay
  - The number of cells grows faster with increasing serum concentration.
- At higher serum concentrations, more cells enter M-phase and divide, leading to a higher growth rate.

Cells Exchange Information and Nutrients With Their Liquid Environment

- Cells die at high concentrations of EtOH
  - Damage to cell membranes
  - Care should be taken with EtOH near cells
- Cells divide more at higher concentrations of serum
  - Growth factors in serum tell cells conditions are good for division
  - Use DMEM with at least 10% serum when high growth rates are desired