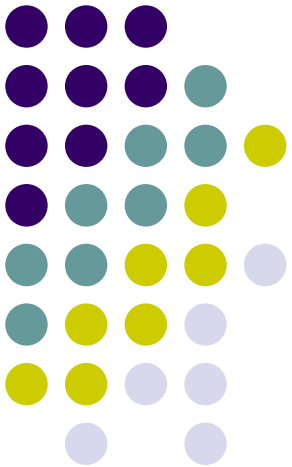
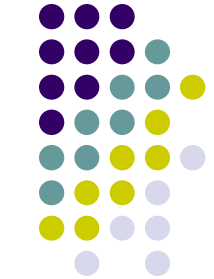
# Assessing the Effects of FBS Concentration on HDF Proliferation

BIOE 342

Spring 2008



# Experimental Objectives

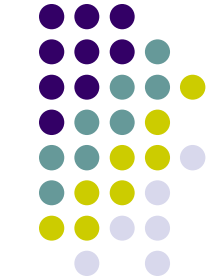


- Relate growth and proliferation of HDF cells to media conditions
  - Test media with 1, 5 and 10% FBS in DMEM
  - Make qualitative and quantitative observations
  - Establish time dynamics of growth rate
- Practice fluorescent and antibody-specific staining techniques

# Proliferation Assessment with Anti-PCNA Staining

- Reveals extent of cell proliferation by binding PCNA, a nuclear protein that is produced during S-phase
- Seed at Day 1: one well per test condition (media containing 1, 5 and 10% FBS), plus 3 wells with 10% FBS for control
- Following two days of incubation, perform assay according to protocol
- Visualize cells using light microscope
  - Red nuclei indicates dividing cell
  - All cells' cytoplasm and non-dividing nuclei stain blue

# Proliferation Assay Reveals Time Dynamics



- Assess extent of cell proliferation by cell concentration at 4 hours and 2, 5 and 7 days after seeding
- Seed Cells
  - 3 wells of each test condition (media containing 1, 5 and 10% FBS) for days 2, 5 and 7 count
  - 6 wells of 1% FBS for 4 hour count
- Determine cell concentration using Coulter Counter for all test conditions at specified time points
- Change media for non-observed cells
- Store cells in incubator during intervals

# Fluorescent Staining Method: Live/Dead Assay

- Stains both live and dead cells
  - Membrane of live cells permeable to green dye
  - Damaged cell membranes permeable to red dye
- On Day 1, seed 9 wells
- Following 2 days of incubation stain 3 wells for each condition (A, B and C)
  - A (live): add 250 µL PBS, 100 µL dye
  - B (dead): add 250 µL ethanol, 100 µL dye
  - C (mix): add 250 µL PBS, 2 drops ethanol, 100 µL dye
- Visualize cells using fluorescent and light microscope

# Results: Extent of Exposure to Ethanol Determines Fluorescence

| Group | Fluorescent Observations | Morphology Observations |
|---|---|---|
| **A** | All cells fluoresce **green** | Elongated - few cells small and circular |
| **B** | All nuclei fluoresce **red** | Elongated and shrunken with condensed nuclei |
| **C** | Some cells fluoresce **green**, some nuclei fluoresce **red** | Some cells elongated, some small and round |

- Viability of cell depends on environment – cells are sensitive to ethanol exposure
- Group A cells killed → **green**
- Group B cells all killed → **red**
- Some group C cells killed → **red** & **green**

# Results: Percent of Cells in S-phase Depends on FBS Concentration

- Anti-PCNA assay indicates cells in 10% FBS experienced the highest level of proliferation at the time of staining
- Relatively little difference in number of dividing cells between 10 and 5% FBS compared to 5 and 1% FBS

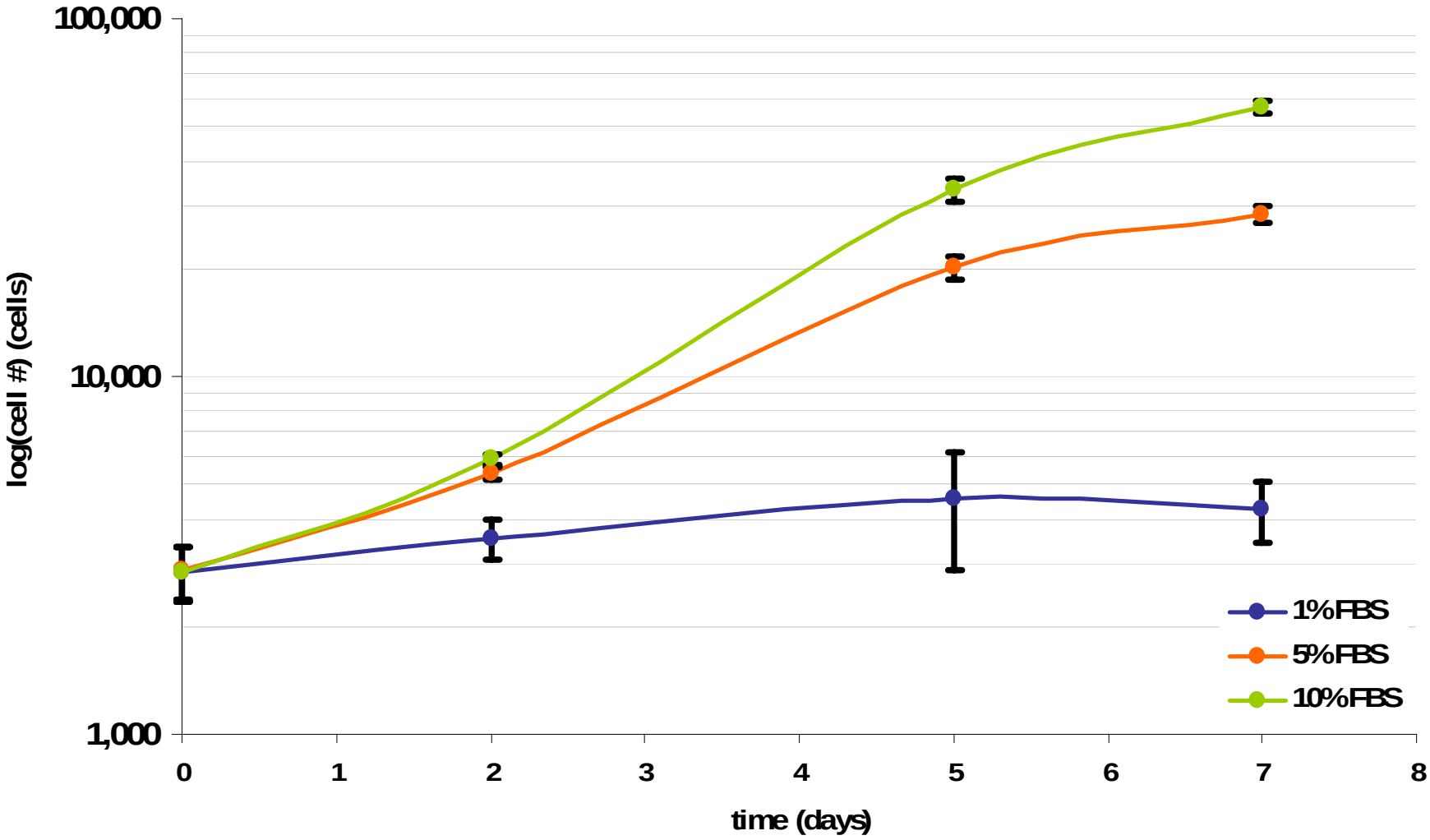
| | **Red-stained nuclei** | **Morphology** |
|---|---|---|
| **1% FBS** | 10% | Stretched with irregular surfaces |
| **5% FBS** | 50% | Elongated |
| **10% FBS** | 60-70% | Very elongated |

# Proliferation Assay: Rate of Cell Growth is Exponential

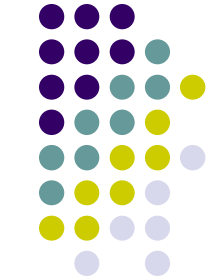
| (total # of cells) | 4 hours | Day 2 | Day 5 | Day 7 |
|---|---|---|---|---|
| **1% FBS** | 2,900 | 3,500 | 4,500 | 4,300 |
| **5% FBS** | - | 5,400 | 20,000 | 29,000 |
| **10% FBS** | - | 5,900 | 33,000 | 57,000 |

- Cells in 10% FBS consistently showed a higher rate of proliferation across all time points
- At day 7 all groups have different cell #s (t-test, $p<0.05$)
- 10% FBS test condition exhibits different cell #s across all time points (t-test, $p<0.05$)
- Doubling time is dependent on % FBS
  - 1% FBS = 8 days 7 hours
  - 5% FBS = 1 day 14 hours
  - 10% FBS = 1 day 5 hours

# Rate of Proliferation Depends on FBS Concentration



# Extent and Rate of Proliferation is Dependent on Media Conditions

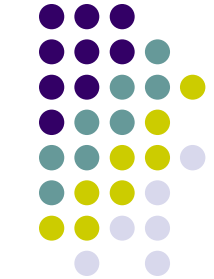


- Proliferation and anti-PCNA assays indicate the highest degree and rate of growth for cells cultured in media with 10% FBS
  - Serum growth factors, lipids and hormones accountable for maximal proliferation
- Cells cultured in 1% FBS exhibit low proliferation rates, which level off after day 2
  - Rate of proliferation limited by availability of serum, causing cells to die

# Staining Offers Advantages in Qualitative Observations

- Live/dead assay - fluorescent visualization offers an advantage over visualization with only a light microscope
  - Live/dead qualifications are difficult to make based on morphology
- Anti-PCNA - staining offers advantages in visulization to determine proliferation
  - Cell phase is very difficult to determine from morphology

# Media Conditions & Proliferation



- Established positive correlation between media FBS content and rate of proliferation in HDF cells
- Highest rate of growth for all conditions experienced between days 2 and 5
- 10% FBS optimal of tested conditions but offers minimal relative advantage in proliferation rate over 5% FBS compared to that over 1% FBS
  - Confirmed by doubling rates