Human Dermal Fibroblasts in Vitro: Viability and Proliferation

Laboratory in Tissue Culture

February 13, 2008

Experimental Objectives

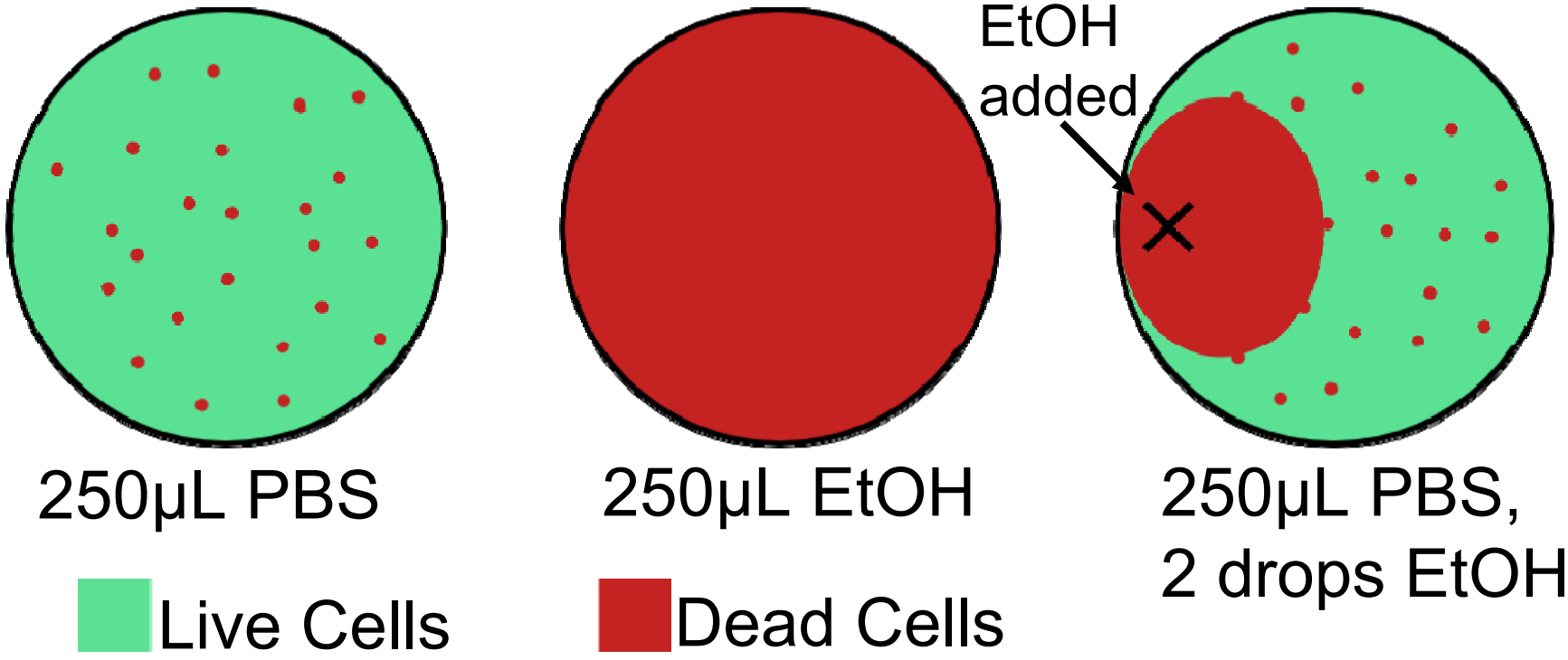
- To qualitatively assess the effects of a toxin, ethanol, on Human Dermal Fibroblast (HDF) cells
  - Live/dead fluorescence reagent used to visualize affected cells
- To examine HDF cell cycle under varying media conditions
  - Anti-PCNA staining used to identify nuclei in S-phase
- To quantitatively measure HDF proliferation rate and its response to varying media conditions
  - Coulter Counter used to determine cell number as time progresses

Effects of Ethanol on Viability: Live/Dead Fluorescence Methods

- HDF cells seeded in TC-treated wells with DMEM, 10% FBS and incubated for 2 days
- Each well rinsed and treated with 1 of 3 conditions:
  - 250 µL PBS
  - 250 µL ethanol
  - 250 µL PBS, 2 drops ethanol
- All conditions treated with fluorescent dyes calcein AM (live cells) and ethidium homodimer (dead cells)
- Light and fluorescent microscopes used to visually assess wells; under a fluorescent microscope, live and dead cells appeared green and red, respectively

Effects of Ethanol: Light vs. Fluorescence Microscopy

- Cells in all conditions displayed similar morphology and degree of attachment under a light microscope.
- The following well patterns were observed upon inspection with a fluorescent microscope:



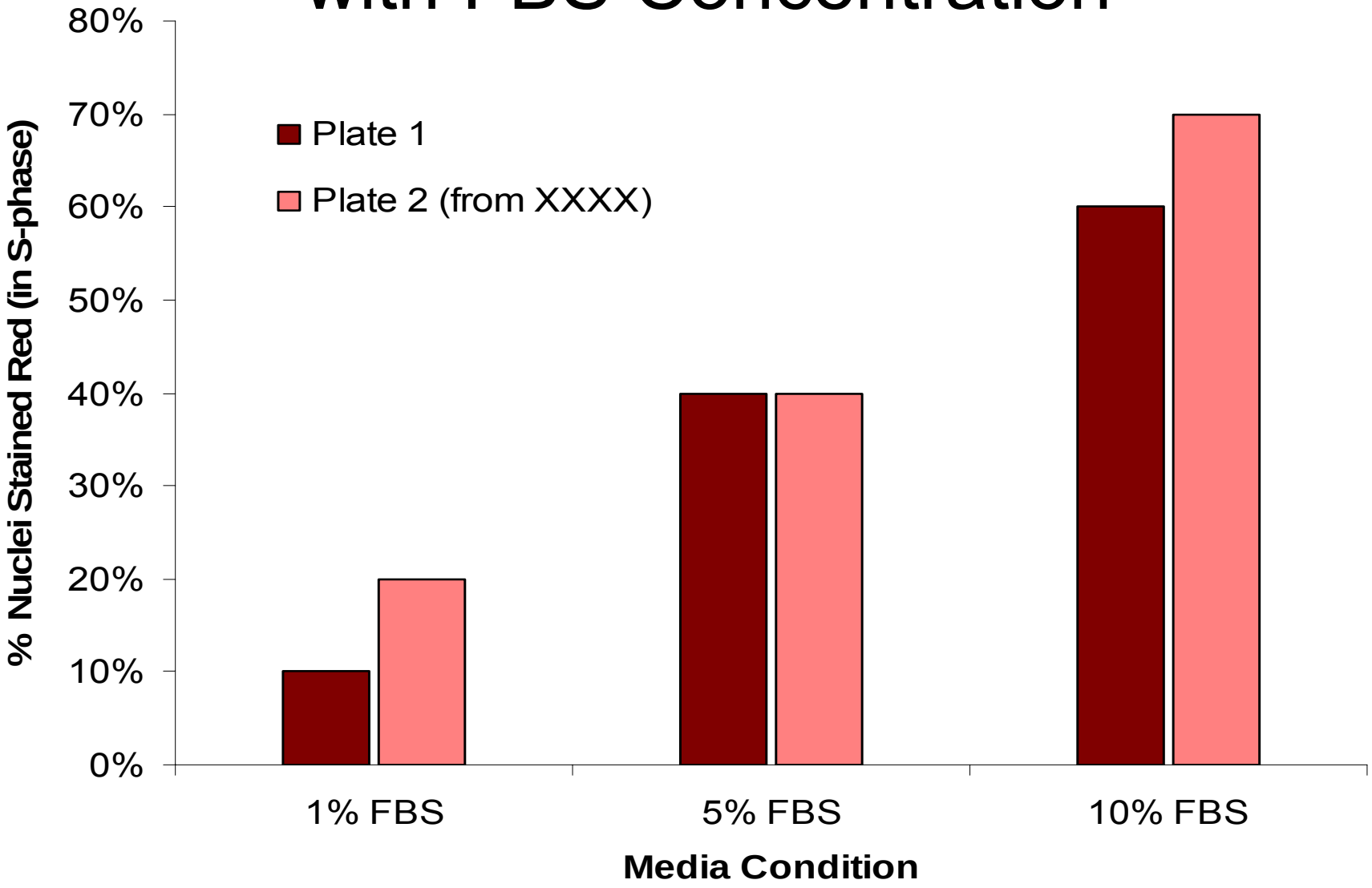
Fluorescence Microscopy: Live/Dead Cell Patterning

- 250 µL PBS
  - Well found to be mostly carpeted with live cells, interspersed with dead cells
- 250 µL ethanol
  - Entire well covered with dead cells; no live cells found
- 250 µL PBS, 2 drops ethanol
  - Live cells interspersed with some dead cells localized on right; area of dead cells found on left, where EtOH was introduced

Effects of Serum on Cell Cycle: Anti-PCNA Staining Methods

- HDF cells seeded in TC-treated wells with DMEM and 1, 5 or 10% FBS; additional controls with 10% FBS prepared
- Cells incubated for 2 days and fixed with formalin
- 1° antibody was used to bind PCNA in S-phase nuclei
- 2° antibody with horseradish peroxidase used to bind 1° Ab and cleave AEC (specific red stain)
- Hematoxylin used for general staining (blue)
- Color of nuclei observed with light microscopy

Fraction of Cells in S-Phase Increases with FBS Concentration

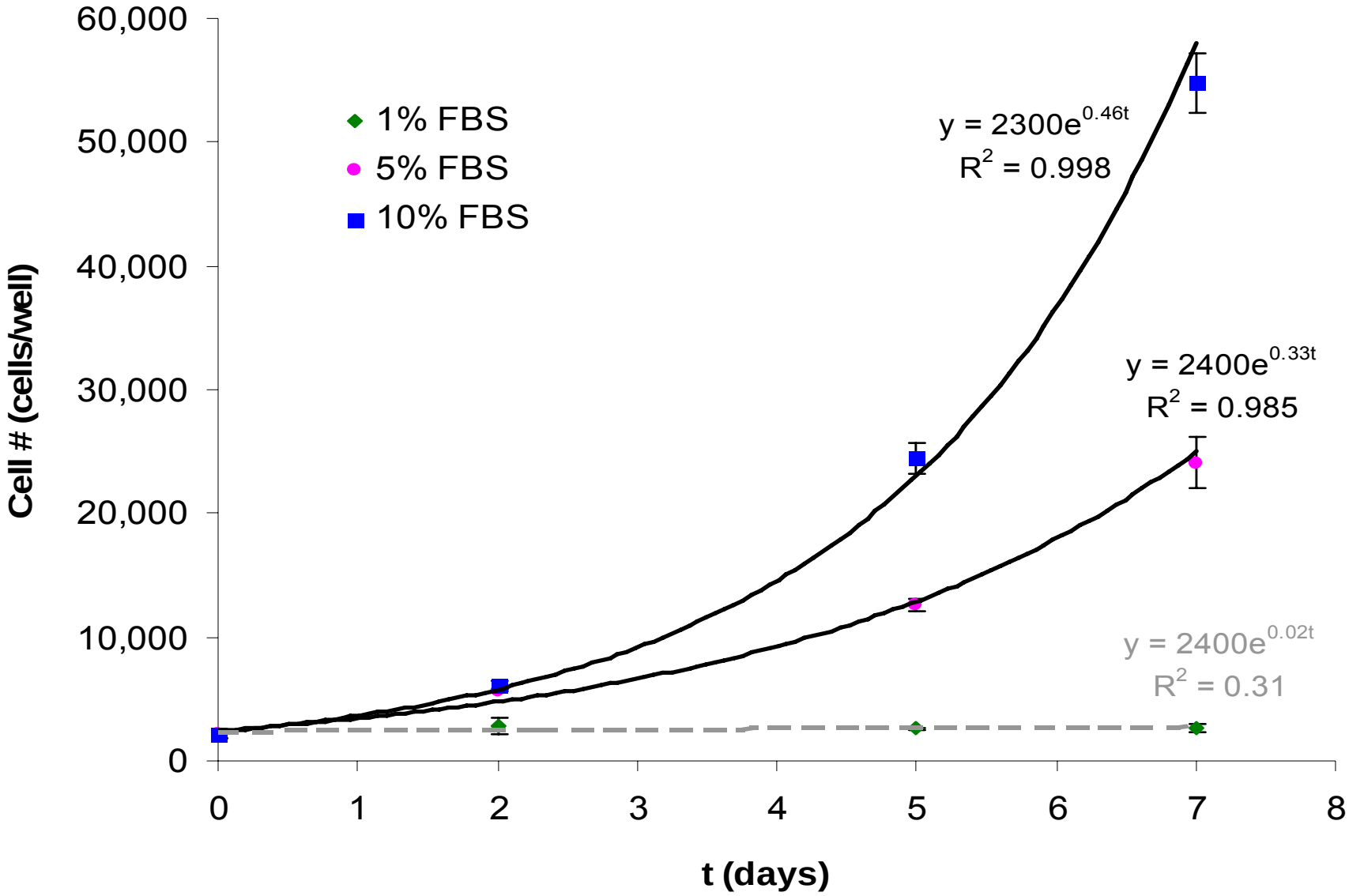


(No S-phase nuclei were found in 10% FBS controls)

Effects of Serum on Cell Replication: Proliferation Assay Methods

- HDF cells seeded in TC-treated wells with DMEM and 1, 5 or 10% FBS
- Cells from each condition trypsinized and rinsed on days 0, 2, 5 and 7
- Cell concentration determined with Coulter Counter
- Number of cells per well calculated for each condition

Cells Exhibit Exponential Growth in 5 and 10% FBS



Cell Number and Doubling Time Vary with Serum Concentration

- Cells in 1% FBS did not display exponential growth.
  - $R^2 = 0.31$
- By day 7, cell numbers in all conditions were statistically different; cell number increased with serum concentration.
  - $p_{1\%,5\%} = 2 \text{ E } -3$
  - $p_{5\%,10\%} = 4 \text{ E } -4$
  - $p_{1\%,10\%} = 4 \text{ E } -4$

Condition	Doubling time
1% FBS	N/A
5% FBS	2.1 days
10% FBS	1.5 days

Cell Growth in Different Media Conditions: S-Phase Cells and Proliferation Rate

- Between the three media conditions tested:
  - Relative number of cells in S-phase increased with FBS concentration.
  - Cell number showed a statistically significant increase with FBS concentration as incubation time progressed.
  - Doubling time shorter in 10% FBS than in 5% FBS; no apparent growth in 1% FBS
- Trends from anti-PCNA staining and the proliferation assay suggest a correlation between fraction of cells in S-phase and proliferation rate.

Summary and Implications

- Ethanol acts as a toxin for HDF cells.
  - Cells survive at lower concentrations; condition with 2 drops ethanol had live cells
  - Morphology and attachment not necessarily affected by ethanol; changes noted with live/dead fluorescent dye
- Fraction of cells in S-phase and proliferation rate increase (i.e. doubling time decreases) as serum concentration increases from 1 to 5 to 10%.
  - possible link between fraction of cells in S-phase and proliferation rate calls for more direct testing of this correlation