Human Dermal Fibroblasts

Techniques to Culture and Assess HDF Cells In Vitro

Assess the survival and function of HDF cells in vitro

- Test for viability
  - Live/Dead Fluorescent Assay: assess the impact of ethanol on HDF cells
- Proliferation
  - Anti-PCNA staining: examine the effect of media conditions on cell cycle and proliferation
  - Cell Proliferation: assess the effects of serum on growth and replication

What is the extent of ethanol's effect ?

- HDF cells dyed with a solution of 2 μM calcein AM and 4 μM EthD-1
- Exposed to three different conditions:
  A. PBS rinse, dye solution (control)
  B. 250 μL ethanol, dye solution
  C. PBS rinse, 2 drops ethanol, dye solution
- Cells examined qualitatively under fluorescent microscope

Do media conditions affect cell cycle?

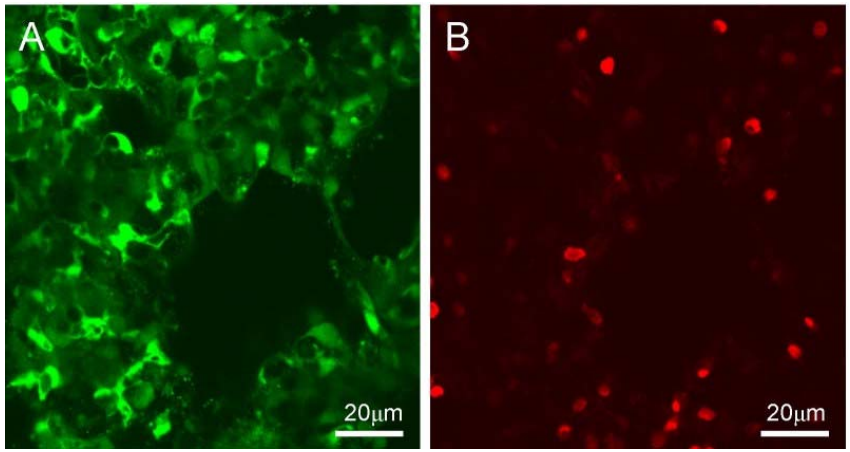
- Anti-PCNA staining
  - HDF cells incubated in DMEM with 1%, 5%, or 10% FBS and 1% antibiotic; fixed in formalin
  - 1° Ab: Anti-PCNA Mouse IgG
  - 2° Ab: Anti-mouse IgG tagged with HRP
  - Two Dyes
    - AEC chromagen – reacts with HRP to stain red
    - Hematoxylin – non-specific, counterstains blue
  - Three Controls
    - Control 1: 1° Ab, buffer
    - Control 2: buffer, 2° Ab
    - Control 3: blocking buffer

Do media conditions affect cell cycle?

- Quantitative Cell Proliferation
  - Day 0: HDF cells seeded (~3250 cells/mL)
  - Cells incubated in DMEM with 1%, 5%, or 10% FBS and 1% antibiotic
  - Days 2, 5, and 7: three wells from each condition were trypsinized
  - Cells counted via Coulter Counter

Fluorescent Assay distinguishes between live and dead cells

Condition	Color	Morphology
A	Green, with sparse red nuclei	Long, thin, and elongated
B	ALL red, no green	Small, rounded nuclei, some cytoplasm visible
C	Red and green present in regions with little overlap	Green cells long and thin, small, round red nuclei



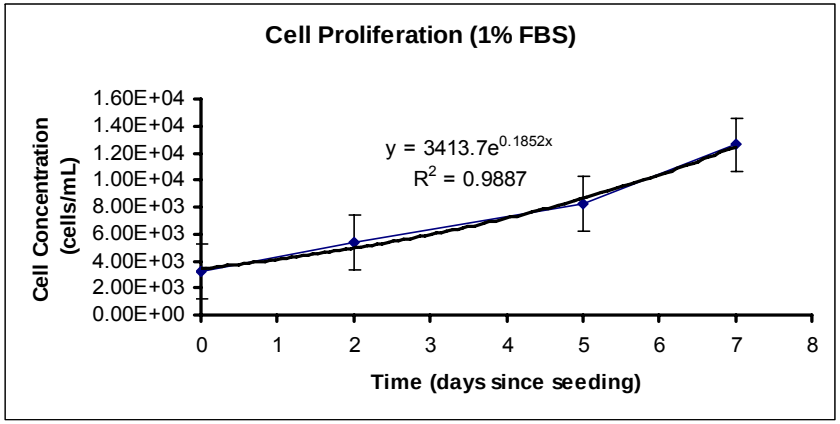
- Fig. A: viable cells stained with calcein AM (488 nm)
- Fig. B: dead nuclei stained with EthD-1 (543 nm)
- All wells were ~95% confluent
- http://bmc.ub.uni-potsdam.de/1465-9921-6-40/F1.htm

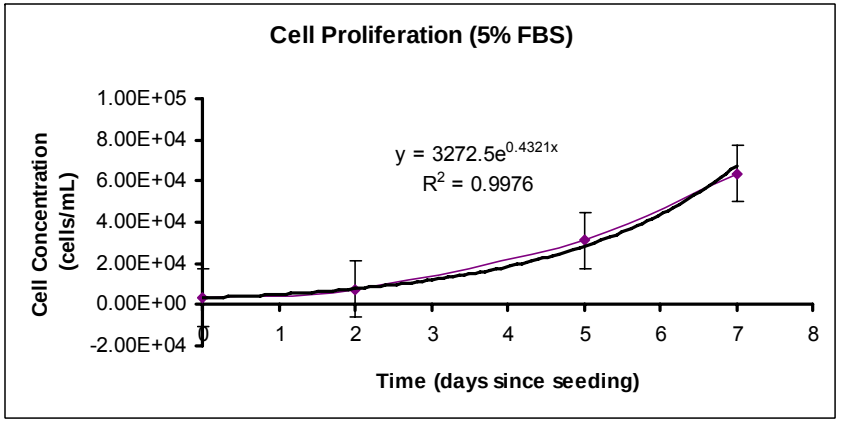
PCNA Assay tags dividing cells

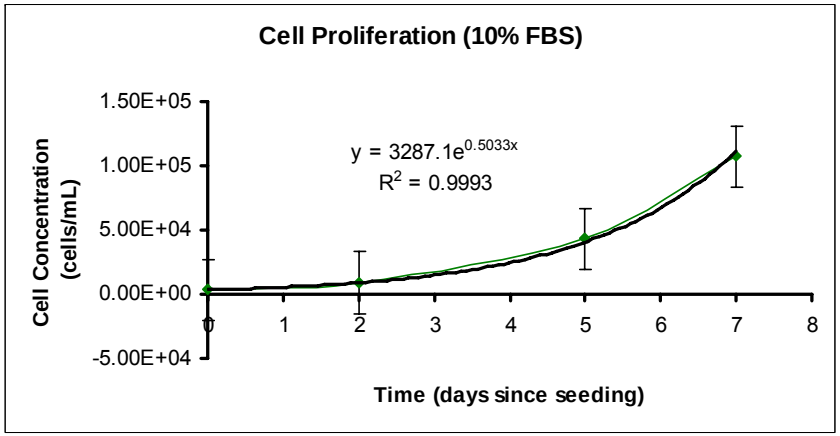
	1% serum	5% serum	10% serum	Control 1	Control 2	Control 3
Confluency	70-85%	90%	80-90%	50-60%	60%	60-70%
Color	25% red 75% blue	40-50% red 50-60% blue	75-80% red 20-25% blue	All cells stained blue	All cells stained blue	All cells stained blue

- Morphology consistent across all wells: long, elongated, with presence of pseudopodia
- More cells undergo cell division at once in higher concentrations of serum

Cell division stimulated by higher serum concentrations

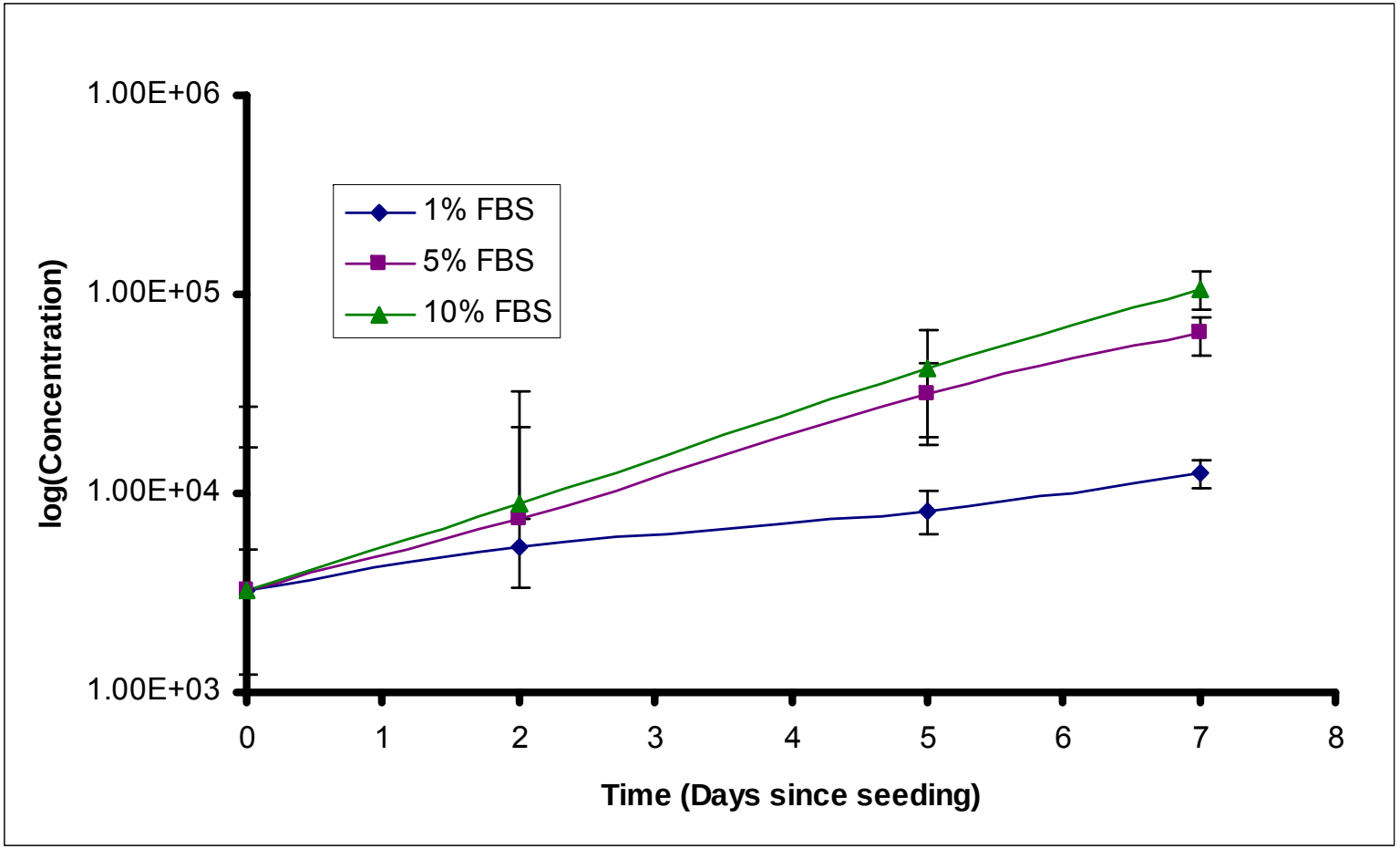






- Cells grow exponentially
- Doubling times:
  - 1% FBS: 3.74 days
  - 5% FBS: 1.60 days
  - 10% FBS: 1.38 days

Higher Serum Concentrations Result in Faster Proliferation Rate



Final cell concentration differs among media types

- The differing growth rates among serum concentrations result in final cell counts that are statistically significantly different from one another.
- 1-Factor ANOVA, $p < 2.56 \times 10^{-8}$

Day 7 Concentrations (cells/mL)

1% serum	5% serum	10% serum
15400	61120	105860
10760	62580	109640
11640	67060	106320

Cell Proliferation consistent with PCNA results

- Two-fold increase in growth rate from 1% to 5% serum
  - Cell Proliferation: Doubling time decreased by 57%
  - PCNA: Fraction of dividing cells doubled
- 10% serum marginally increases the growth rate
  - Cell Proliferation: only represents 15% increase
  - PCNA: Fraction of dividing cells increases by 50%

Key Results

- Live/Dead Fluorescence Assay a useful technique for determining cell viability post-exposure to toxic substance
- Cells exhibit exponential growth
- Increasing serum concentration increases rate of proliferation due to presence of extra growth factors and nutrients
- Increasing concentration past certain point has no effect – cells reach saturated rate of division