Viability and Proliferation of Human Dermal Fibroblasts *in vitro*

Spring 2008

Objectives

- To develop a relationship between MTT assay absorbance results and cell counter values
- To measure proportions of dividing to non-dividing cells under different media conditions
- To observe cell proliferation over time, and analyze relative to anti-PCNA staining results

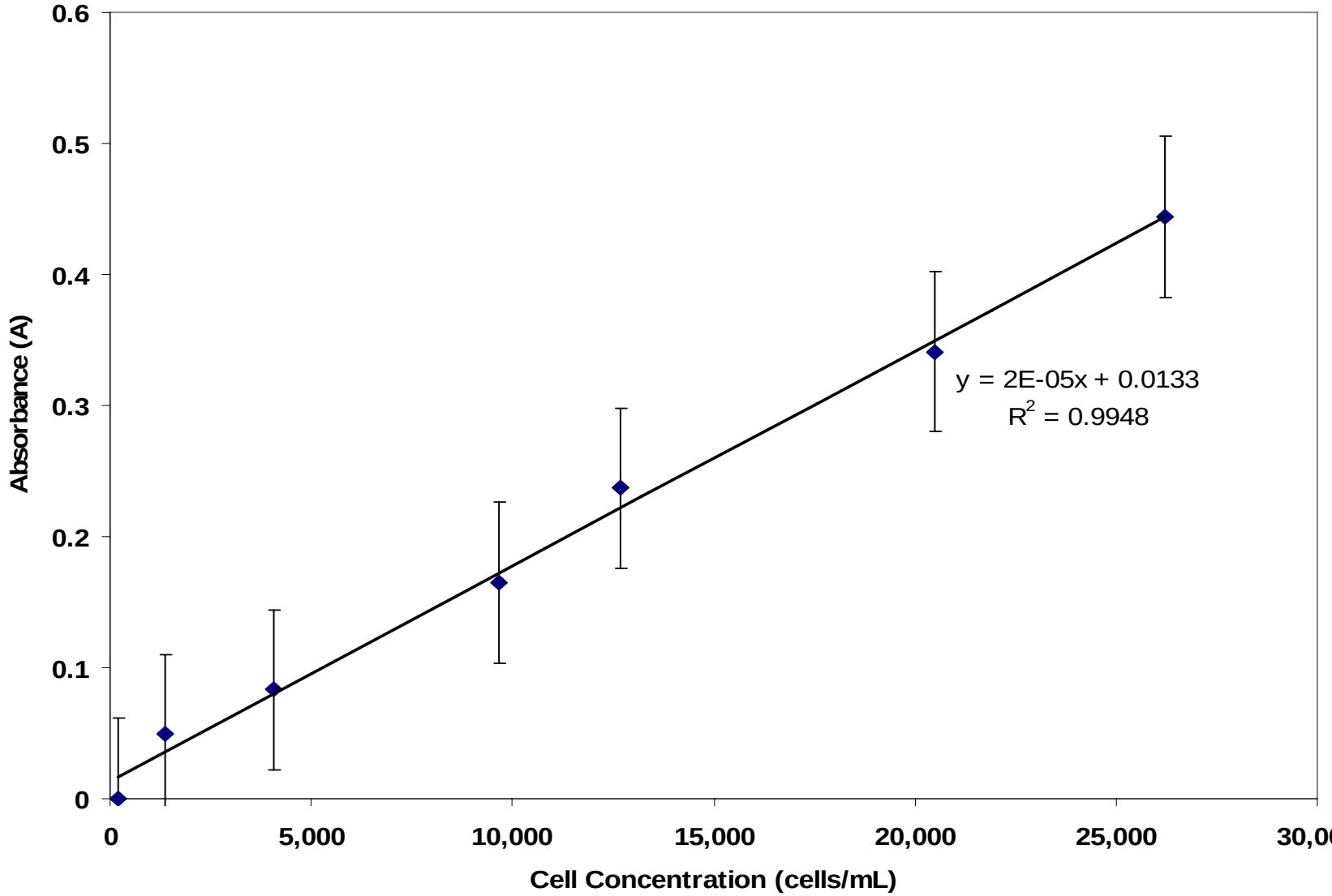
Experimental Methods

- MTT viability assay
  - Cells seeded at varied concentrations into 24-well plates; two wells of each concentration
  - One set of wells of each concentration treated with MTT dye. **Absorbance** of each sample was measured on a Genesys 10UV spectrophotometer.
  - **Concentrations** of the control wells determined via Coulter Counter

Experimental Methods

- Anti-PCNA staining assay
  - Cells seeded into a 24-well plate; incubated under varied media conditions.
  - Fixed with formalin; stained with anti-PCNA primary antibody, HRP secondary antibody, and a chromogen. **Ratios of red-stained (dividing) to blue-stained (nondividing) cells** were observed under a light microscope.
- Cell proliferation assay
  - Cells seeded into 24-well plates, allowed to attach, incubated under varied media conditions (1,5, or 10% serum)
  - Cells trypsinized and counted via Coulter Counter at 4 hours, and 2, 5, and 7 days for each media condition. **Cell number** was plotted over time for each condition.

MTT Viability assay: absorbance and concentration



MTT viability assay: results summary

- Absorbance at 570 nm of MTT-dyed cell suspensions shows a linear relationship to cell count determined by Coulter Counter.
- Linear fit (A = absorbance; C = counted cell concentration): $A = (2 \times 10^{-5})C + 0.013$
- More tests necessary to establish a definite relationship between absorbance and **viable** cell concentration

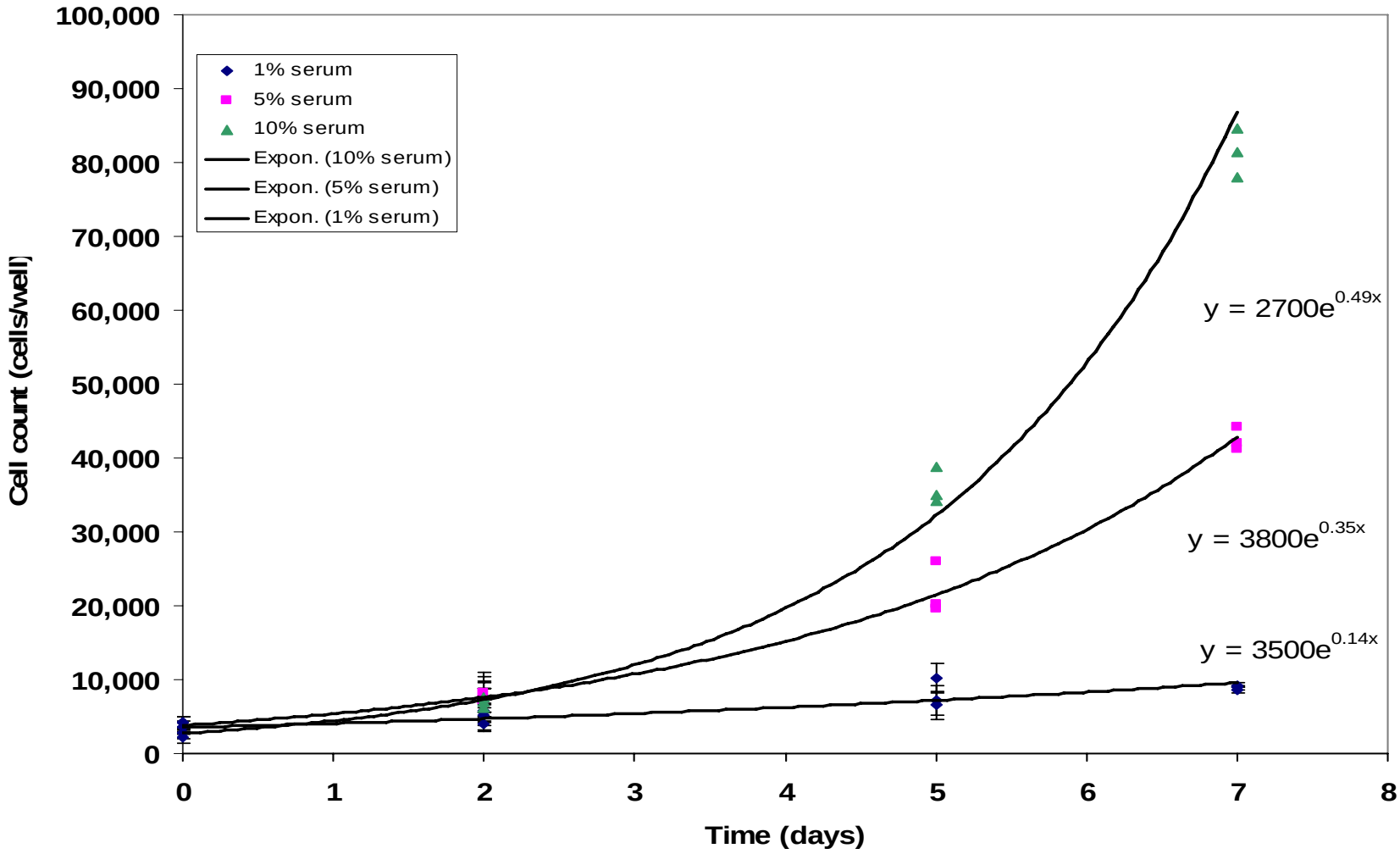
Anti-PCNA staining assay for cell division

% serum in media	Dividing (red): nondividing (blue) ratio
1%	1:9
5%	3:7
10%	7:3

Controls had only one or neither antibody, and all showed only blue nuclei (nondividing indicator).

- Results are approximate; only one sample for each condition
- The highest proportion of dividing cells was observed in the 10% serum condition; lowest in the 1% condition.

Cell proliferation assay: growth over seven days



Cell proliferation assay: doubling times for varied media conditions

% serum in media	Approximate cell doubling time (days)
1%	4.9
5%	2.0
10%	1.4

Cell proliferation assay: results summary

- Cell doubling time was shortest in 10% serum media, longest in 1% serum media.
- Cell number means between time points are statistically significantly different from one another (ANOVA, $p<0.001$).
  - All pairwise comparisons of time points also yield significant differences (ANOVA, Tukey, $p<0.05$).
- Cell number means among conditions on day 7 are statistically significantly different from one another (ANOVA, $p<0.001$).
  - All pairwise comparisons of conditions also yield significant differences (ANOVA, Tukey, $p<0.05$).

Anti-PCNA and cell proliferation assays: comparing results

- Anti-PCNA staining shows relative numbers of dividing and nondividing cells.
  - Indicator of cell proliferation speed; more cells dividing → faster population growth overall
- Proliferation assay indicates doubling speed is faster for 10% serum than for 5% or 1%.
- Results agree between the two assays.
  - Anti-PCNA shows most dividing cells in 10% serum media; implies fastest population growth and thus agrees with cell proliferation assay.

Summary/Conclusions

- MTT assay: linear relationship between dyed cells' absorbance at 570nm can be modeled by [$A = (2 \times 10^{-5})C + 0.013$].
- Cells grown in media with 10% serum have a higher proportion of dividing cells than those grown in 5% or 1% serum media.
- Cells grown in media with 10% serum exhibit a faster population doubling time than those grown in 5% or 1% serum media.