

Conditions for Cell Attachment and Proliferation

BIOE 342

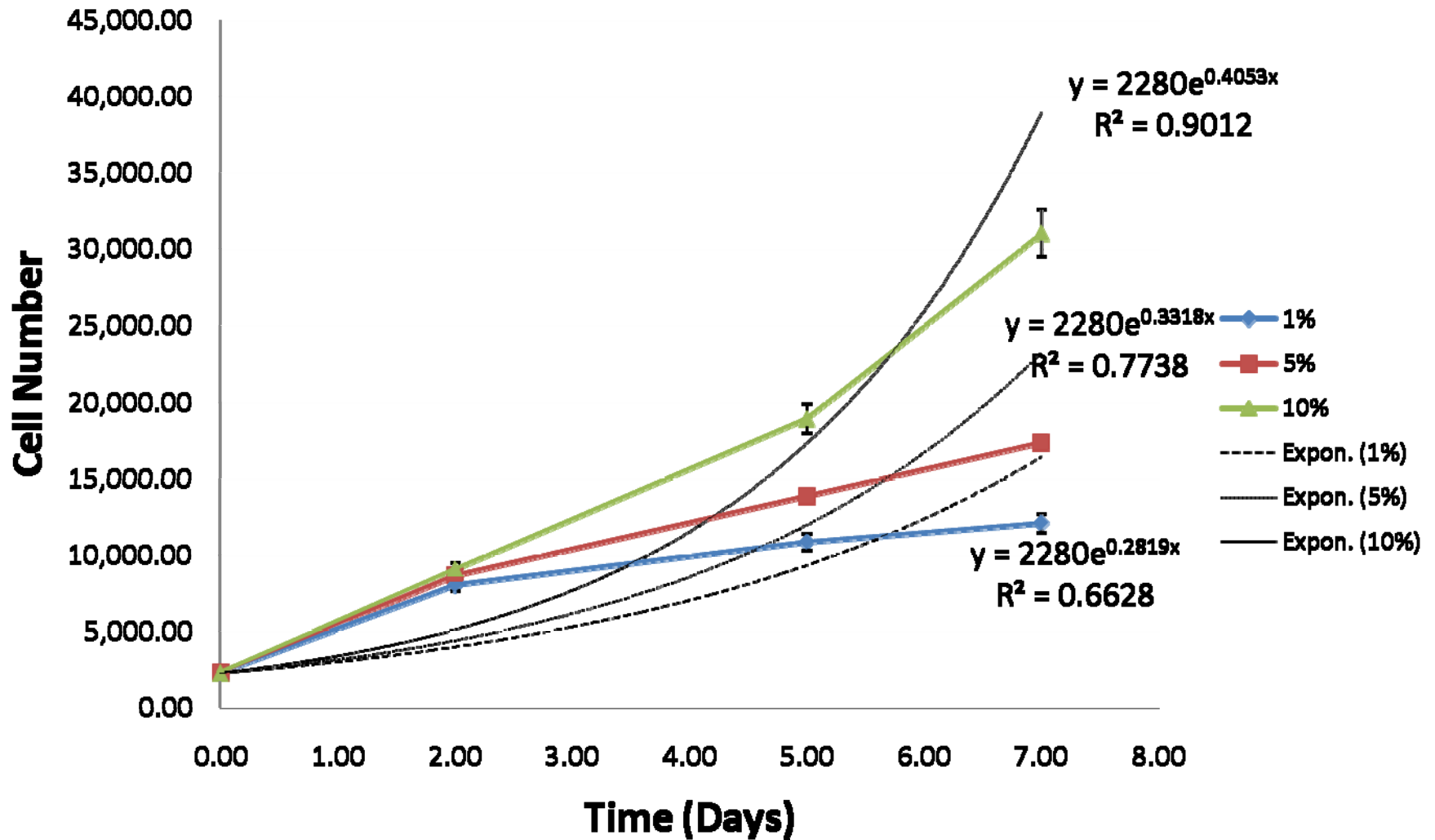
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Purpose & Objectives

- **PURPOSE:** To use standard lab protocols in order to determine the effects of different conditions with regards to cell growth and proliferation.
- **OBJECTIVES:** To become familiar with different assays and their techniques, including PCNA staining, as well as the use of a Coulter Counter and different types of plates.

Cell Proliferation Assay



Cell Proliferation Assay

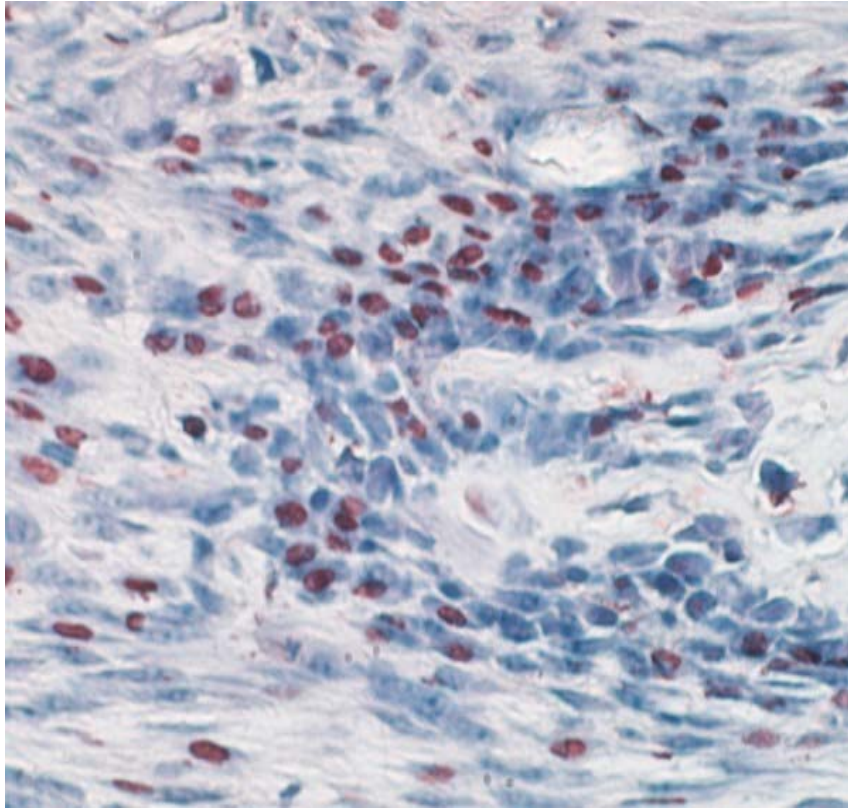
METHODS

- Treated tissue culture plates, micropipettes, DMEM at various concentrations.
- Seeded wells at 1%, 5% and 10% serum concentrations.
- Coulter Counter determinations conducted at days 0-4 hrs, 2, 5 and 7.

RESULTS

- The higher the serum concentration, the faster the rate of growth.
- The lag phase is approximately two days, with most growth between 2-5 days, with a plateau around day 7.
- Cells grow exponentially.

Anti-PCNA Assay



http://www.cor.uams.edu/images/youngmouse_do%20pcna40x.jpg

METHODS

- Anti-PCNA with horseradish, AEC solution, FBS, formalin, TBS hematoxylin, light microscope.
- Seeded wells at 1%, 5% and 10% serum concentrations and before staining.
- Viewed under a light microscope for results.

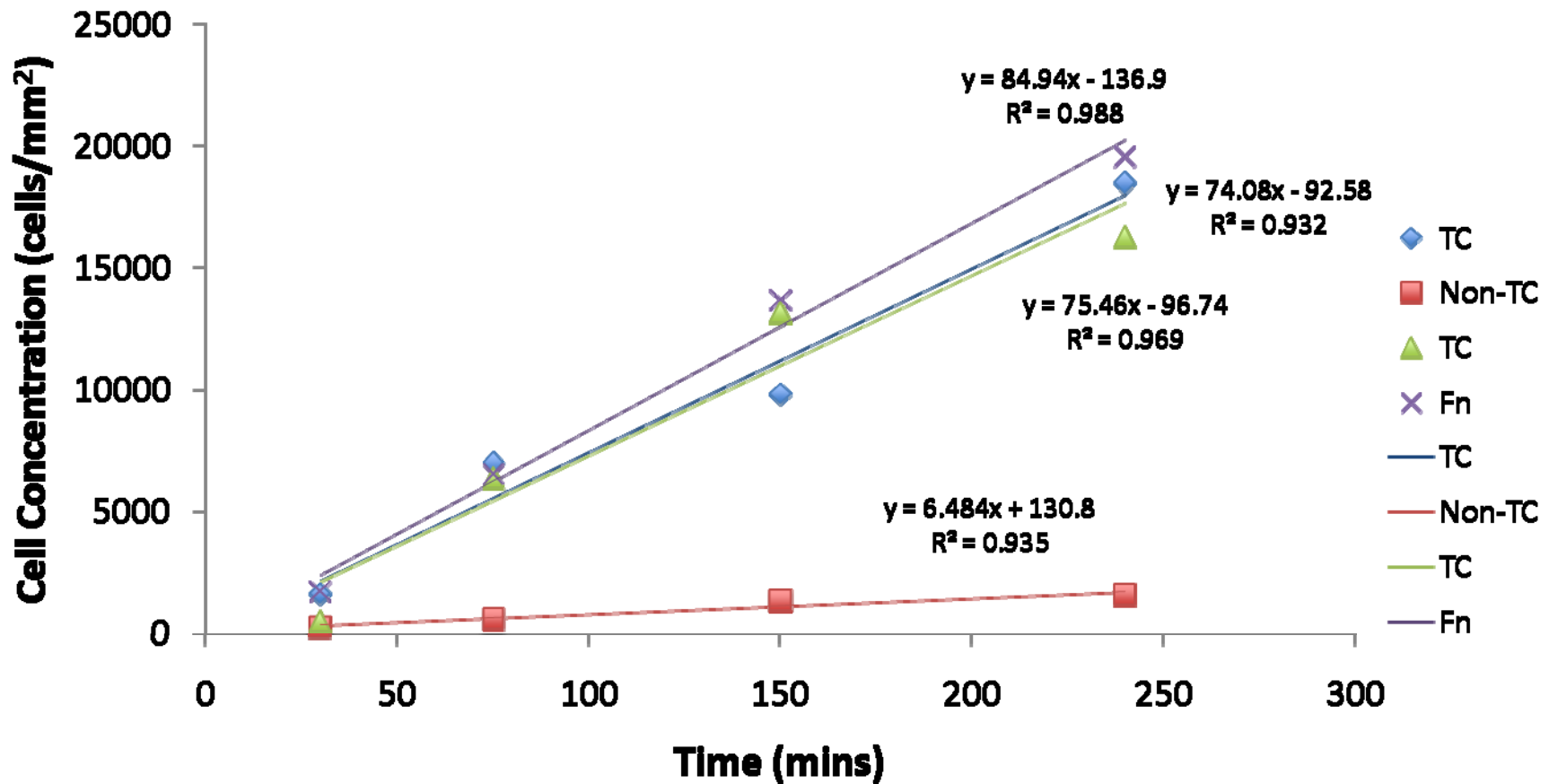
Anti-PCNA Assay

RESULTS

- Cell nuclei stained red indicated that they were undergoing division.
- Cell nuclei stained blue indicative of cell stability.
- The higher the serum concentration, the more red stained cell nuclei present.
- Serum concentration has a direct correlation to cell growth and division.

Quantitative Cell Attachment Assay

Rate of Quantitative Cell Attachment



Quantitative Cell Attachment Assay

METHODS

- 10% DMEM, Fn, TC, n-TC plates, light microscope.
- Seeded wells for each plate type.
- Cell counts conducted at 30 mins., 1 hr. 15 mins, 2 hrs. 30 mins., 4 hrs.

RESULTS

- The n-TC plate had the least number of attached cells.
- The Fn plates had the best rate of attachment.
- Cells need a minimum of four hours in order for widespread cell attachment to occur.

Results

- HDF cells grow best in 10% DMEM.
- Confluency is reached in approximately 7 days under optimal growing conditions.
- The higher the serum concentration, the faster the cells proliferate.
- Fn plates allow more cells to attach than any other plate type.
- It takes about four hours for most cells to attach.

Discussion & Further Study

- What are other ways to manipulate the rate of cell growth?
- How might these results change with other cell types and cell lines?
- What results might you get if you extended the quantitative cell attachment assay to a week?
- At what point and under what conditions will the cells start to die?