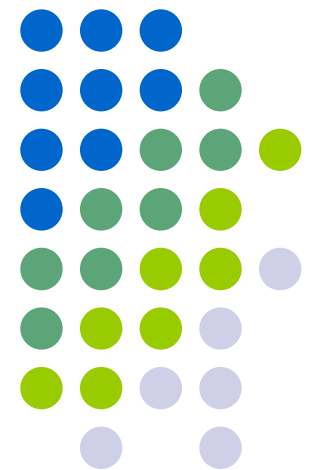
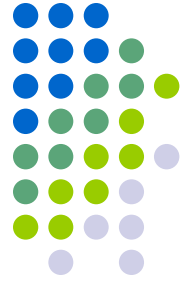


Measuring HDF Cell Viability and Proliferation

BIOE 342 – Tissue Culture

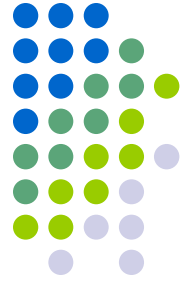


Purpose



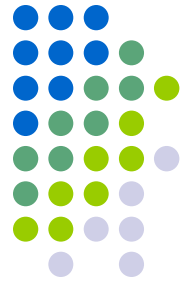
- To find a simple method of analyzing HDF cell viability
 - MTT Assay relates cell viability to cell solution color
- To examine how serum percentage affects HDF cell proliferation
 - Anti-PCNA staining uses nucleus color to determine cell ability to proliferate in different serum percentages
 - Cell proliferation assay measures cell concentration over time in response to different serum percentages

Cell Viability Indicated by Color of Solution in MTT Assay



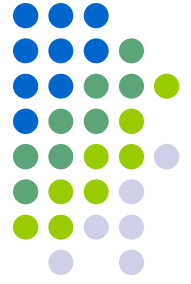
- Cells seeded on two plates at stock concentration and 5 dilutions of stock: 1:1.5, 1:2, 1:3, 1:6, and 1:12, then incubated for 2 days at 37°C and 5% CO₂
- One plate Coulter counted for cell concentration data; other plate was stained with yellow MTT dye to determine viable cell concentration.
 - Viable cells metabolize the MTT dye, changing it from yellow to purple.
- Absorbance of stained cell solution measured by a Genesys 20 Spectrophotometer and compared to Coulter counted cell concentration

Anti-PCNA Staining Indicates Cells in S-phase



- Cells seeded at uniform concentration in 1%, 5%, and 10% serum and incubated for 2 days at 37°C and 5% CO₂; positive and negative controls also seeded
- Cells incubated with anti-PCNA antibody and secondary antibody with horseradish peroxidase to prevent non-specific binding
- Cells incubated with AEC and hemotoxylin
 - AEC dyes cell nuclei in S-phase red.
 - Hemotoxylin stains all parts of the cell blue.
- Percentage of stained cells able to proliferate determined with a Nikon light microscope

Proliferation of Cells Over Time in Response to Serum Percentage

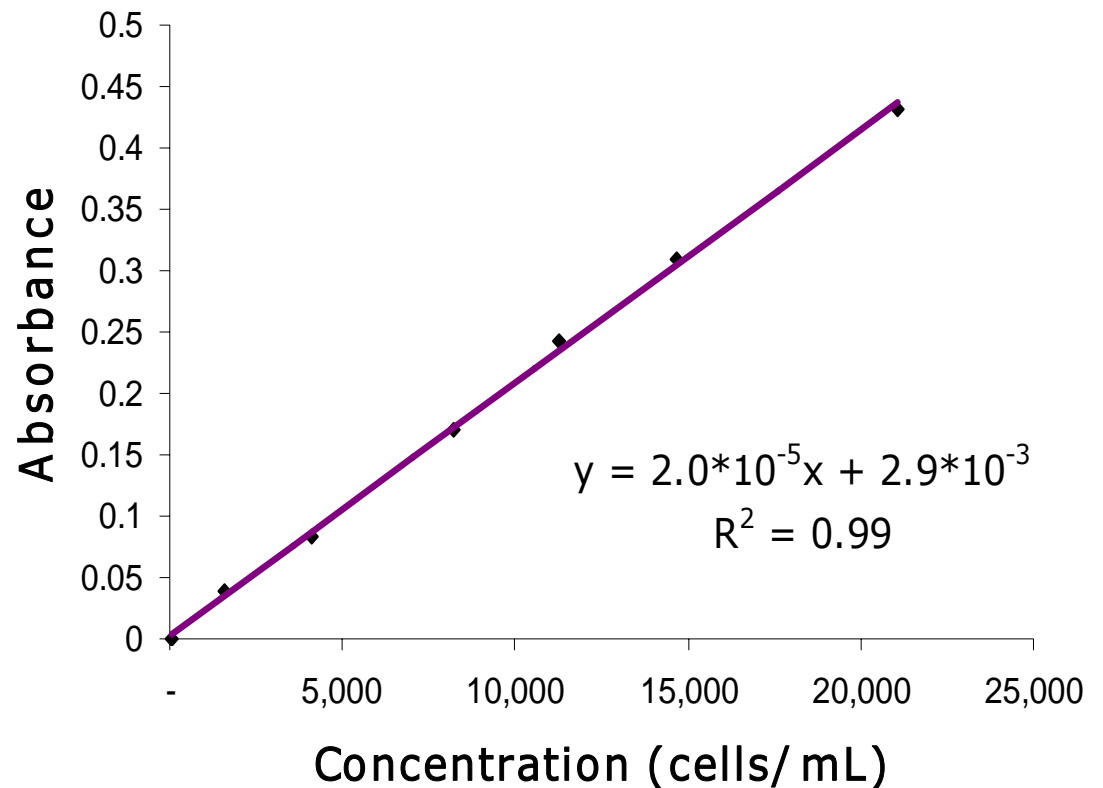


- For the cell proliferation assay, cells were seeded at uniform concentration for examination on Days 0, 2, 5, and 7 and incubated at 37°C and 5% CO₂.
- For each day condition except Day 0, cells were cultured in either 1%, 5%, or 10% serum.
- On appropriate day, trypsin was used to remove attached cells; cells were then Coulter counted to determine difference in concentration and proliferation based on serum percentage.

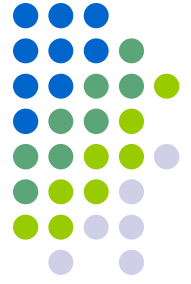
Greater Viable Cell Concentration Indicated by Greater MTT Absorbance



- Greater measured absorbance with higher cell concentrations
 - Larger amounts of viable cells in high cell concentration converted MTT dye from yellow to purple.
- Linear relationship observed between cell concentration and absorbance ($R^2 = .99$)

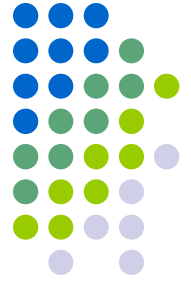


Proliferation Ability Increases with Increasing Serum Percentage and Decreasing Confluency



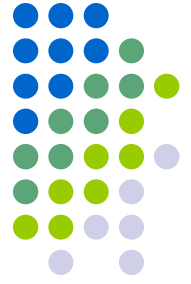
- Number of cell nuclei in S-phase increases as serum percentage increases
 - 10% serum concentration produces maximum percentage of red nuclei in both high and middling confluencies.
- As confluency decreases, number of nuclei in S-phase increases
 - Comparing identical serum conditions, cells with lower confluency had greater percentage of red nuclei.
 - This is because cells in lower confluency conditions have space to proliferate.

HDF Cells in 10% Serum Have Greater Ability to Proliferate



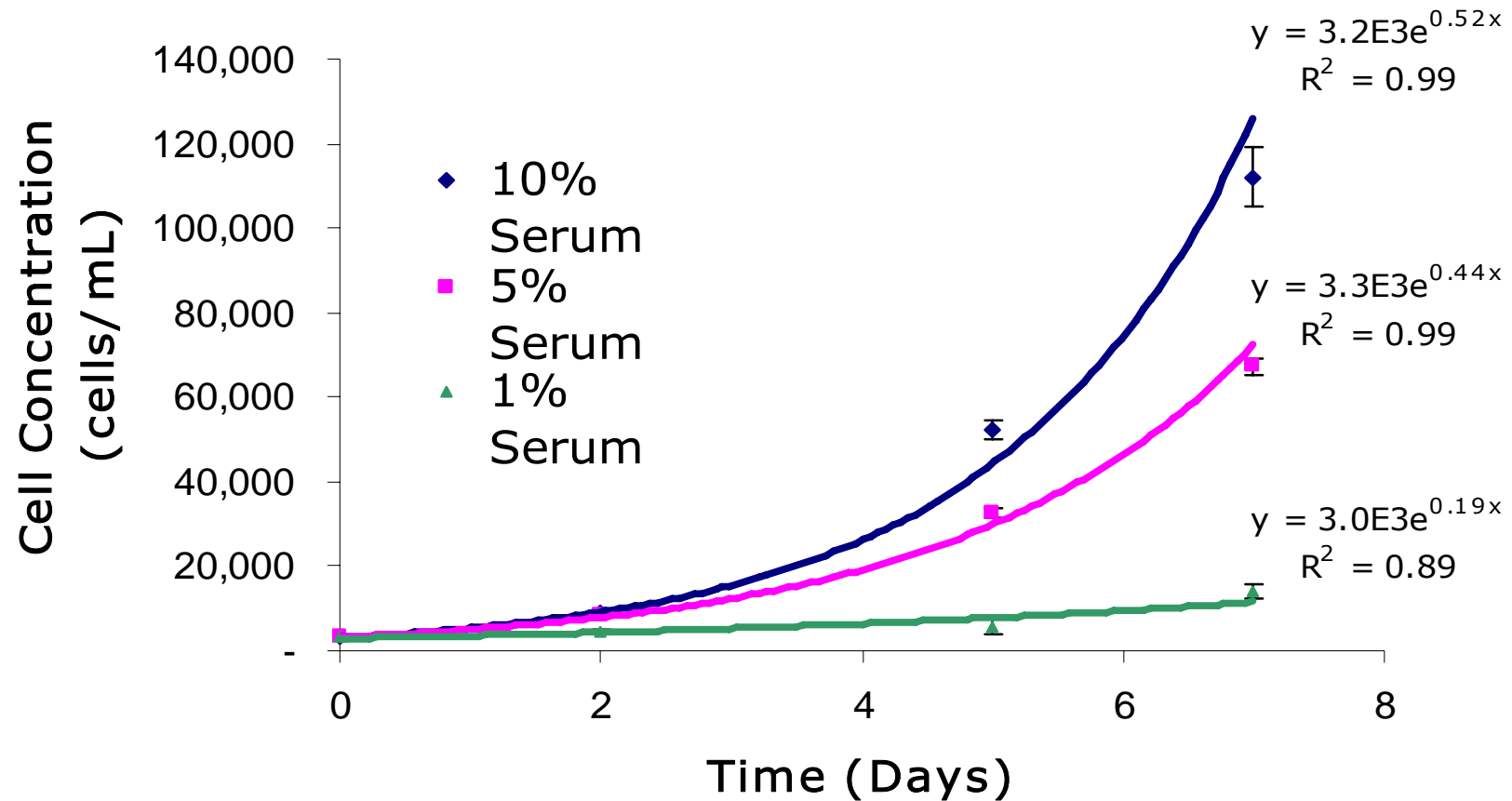
Confluency	Serum Percentage	Percent Red Nuclei	Percent Blue Nuclei
High (> 80%)	10%	50%	50%
	5%	30%	70%
	1%	20%	80%
Middling (40-80%)	10%	75%	25%
	5%	60%	40%
	1%	40%	60%

Greater Serum Percentage Leads to Greater HDF Cell Proliferation

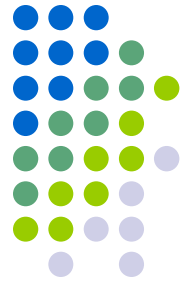


- As serum percentage increased, so did proliferation of HDF cells.
 - 5% serum induced greater proliferation than 1% serum (Student's t-test, $p < .05$).
 - 10% serum induced greater proliferation than 1% and 5% serum (Student's t-test, $p < 0.5$, $p < .05$)

Greater Serum Percentage Leads to Greater HDF Cell Proliferation

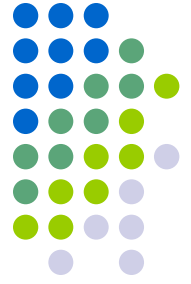


Comparison of Proliferation Assay Results



- Both assays measure cell proliferation or ability to proliferate in response to 1%, 5%, and 10% serum conditions.
 - Cell proliferation assay quantitatively measures proliferation by determining cell concentration
 - Anti-PCNA staining qualitatively measures cell ability to proliferate by estimating number of cells in S-phase
- In both assays, 10% serum is the ideal serum condition tested for cell proliferation.
 - Cell proliferation assay in 10% serum had highest final cell concentration of conditions tested
 - Anti-PCNA staining in 10% serum had greatest number of cells in S-phase in both high and middling confluencies compared to other conditions tested

Conclusions



- MTT Assay simple and useful way to determine viable cell count through spectrophotometry
 - Linear curve can be used to determine MTT-dyed cell concentrations without Coulter counter in future.
- 10% serum is optimal percentage of serum for HDF cell proliferation among conditions tested
 - In cell proliferation assay and anti-PCNA staining, 10% serum was shown to increase final cell concentration and increase number of cells able to proliferate, respectively.