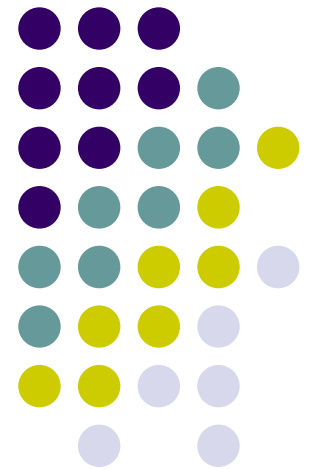
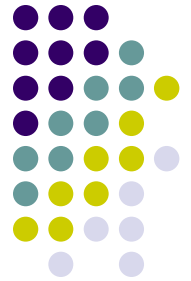


Cell attachment and proliferation under various conditions

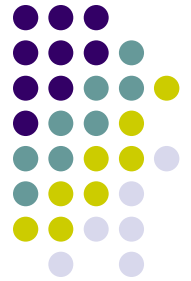
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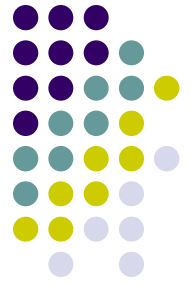
Objectives

- Investigate the relationship between cell attachment and surface conditions
- Visualize the effects of different serum concentrations on cell proliferation
- Quantitatively assess proliferation over time under varying serum concentrations



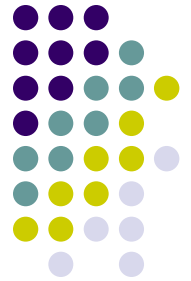
Measuring cell attachment

- 10,000 cells/mL suspensions prepared in DMEM with 10% FBS
- 1 mL cells seeded into each of 12 wells on:
 - TC treated
 - non-TC treated
 - Fibronectin treated 24 well plate
- Cells in 1 mm² of each of three wells manually counted under light microscope at 30 min, 1:15, 2:30, and 4:00 hours after incubation



Visualizing cell proliferation

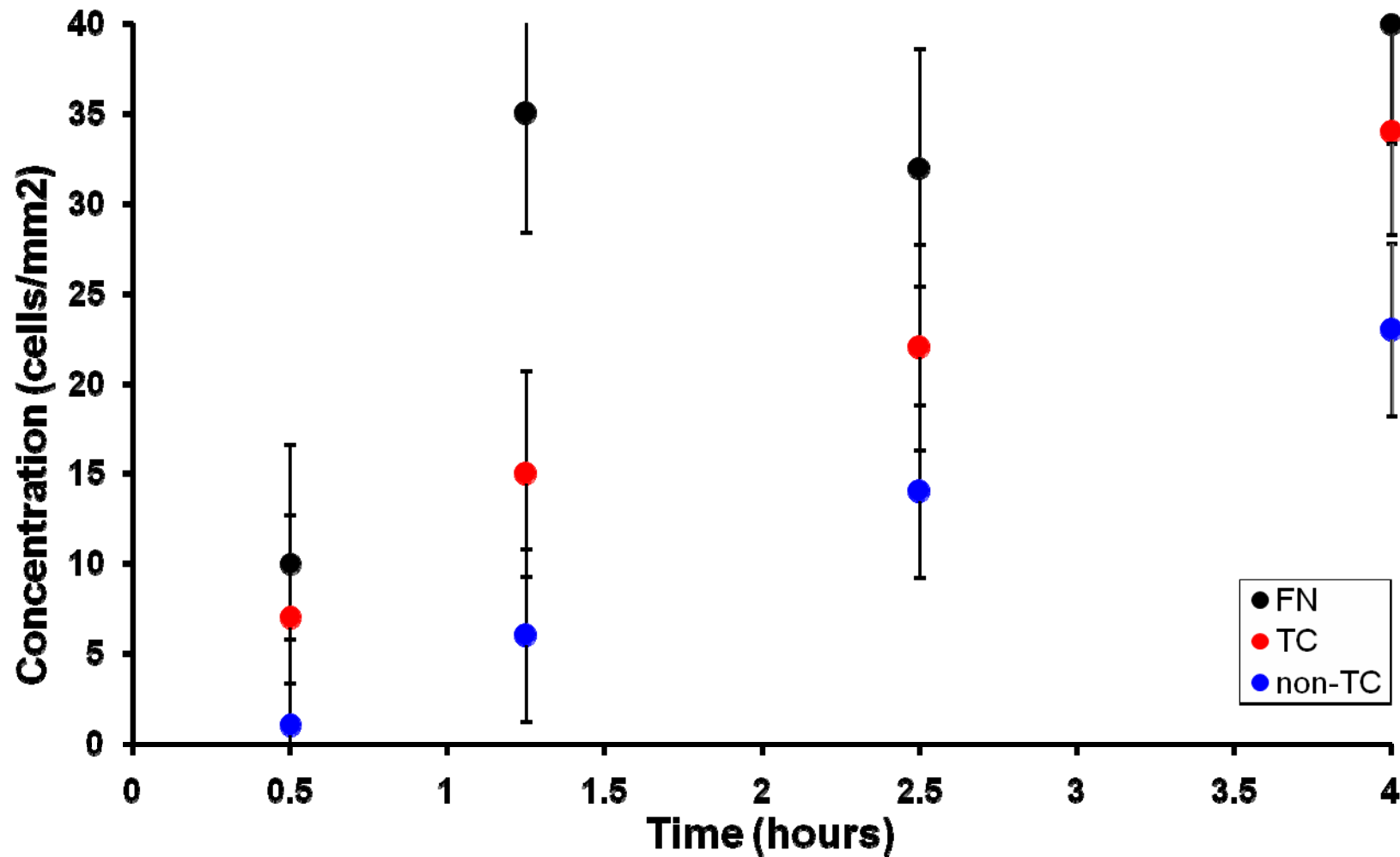
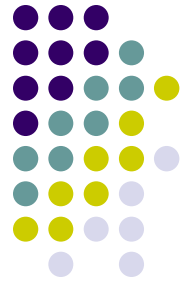
- 20,000 cells/mL suspensions prepared in DMEM with 1%, 5%, and 10% FBS and seeded into 3 wells on TC treated 24 well plate
- After 2 days incubation, experimental wells treated with anti-PCNA antibody and stained with AEC solution and hematoxylin
- Nuclei of cells in S phase stain red
- Percentage of cells committed to divide estimated by eye under light microscope



Tracking cell proliferation

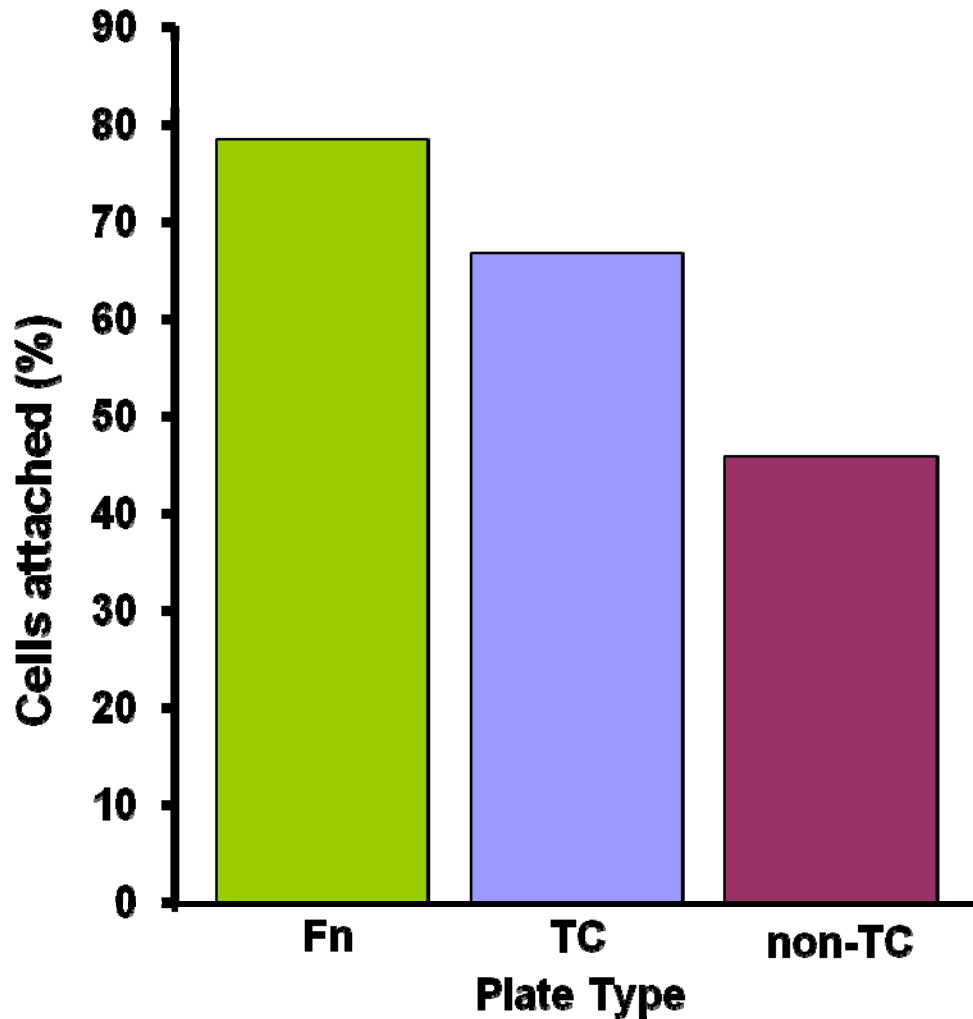
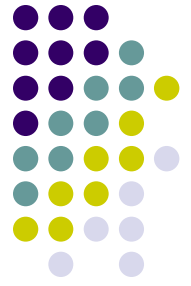
- 5,000 cells/mL suspensions prepared in DMEM with 1%, 5%, and 10% FBS
- Wells on TC treated 24 well plate seeded with three samples of each suspension, for 4 total days of measurement, then incubated
- 3 wells per sample trypsinized and counted with Coulter Counter at 0, 1, 3, and 6 days after incubation

Cells attach best to fibronectin coated plate



Data for fibronectin coated plate collected by Alex Siller and data for TC treated plate averaged with data collected by Alex Siller

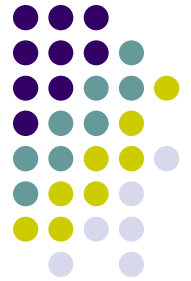
Greatest attachment on TC treated plate after 4 hours



- Initial cell count 10,200 cells/well
- n = 3 samples
- Fn > TC (T-test, $p < 0.01$)
- TC > non-TC ($p < 0.01$)

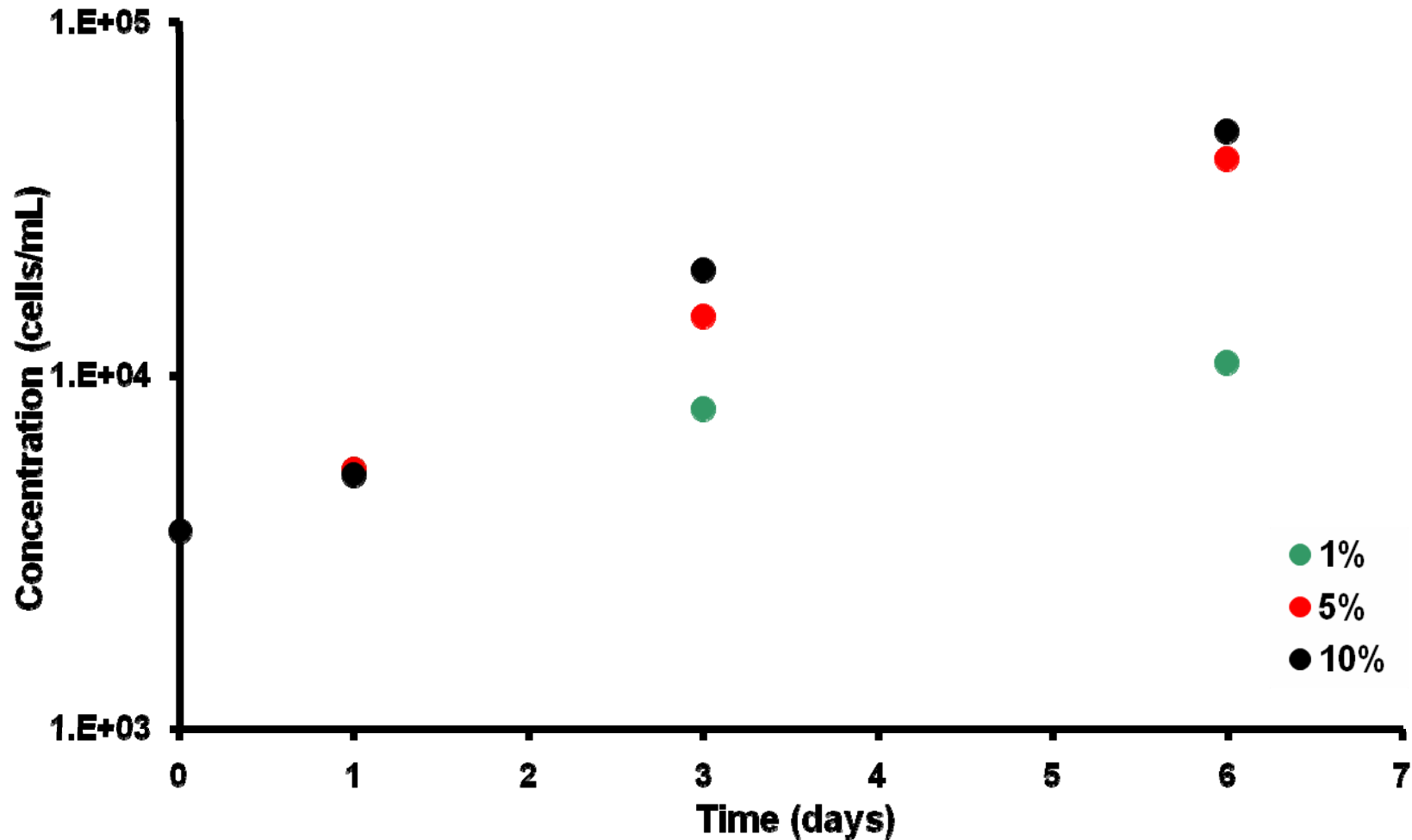
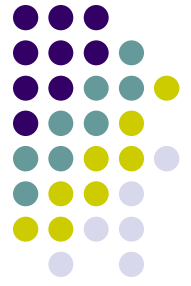
Data for fibronectin coated plate collected by Alex Siller and data for TC treated plate averaged with data collected by Alex Siller

Serum increases cell proliferation

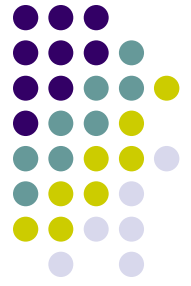


% FBS in DMEM	Observations
1%	Blue cells, dark blue nuclei, 30% red nuclei
5%	50% red, half of cells in S phase
10%	80% red nuclei, 20% not in S phase

Serum promotes cell proliferation over time



Fastest cell doubling time in 10% media



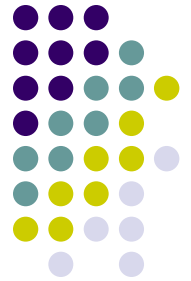
Doubling Time (days)	
1%	5.1
5%	1.7
10%	1.5

- $$T_d = (t_2 - t_1) * \frac{\log(2)}{\log\left(\frac{q_2}{q_1}\right)}$$

www.wikipedia.org

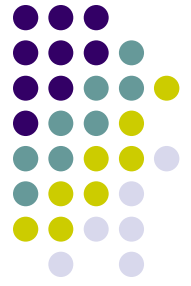
- Calculated over 5 day data range ($t_2 - t_1$)
- Lowest doubling time indicates fastest proliferation rate

10% FBS better than 5% or 1% for cell proliferation



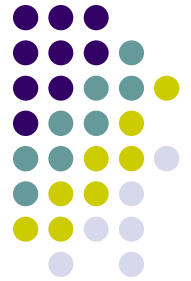
- Anti-PCNA assay
 - Most cell nuclei stained red
 - Dividing cells most prevalent
- Proliferation assay
 - Greatest proliferation rate
 - Lowest doubling time

Fibronectin treated plate better for cell attachment



- Greatest percentage of cells attached
- No significant difference in attachment rate (T-test, $p > 0.05$)

Conditions for optimal cell attachment and proliferation



- Fibronectin coated plate
 - Attachment rate inconclusive
 - Greatest percentage of attached cells in comparison to TC and non-TC plates
- DMEM with 10% FBS
 - Greatest amount of cell proliferation
 - Greatest proliferation rate in comparison to 1% and 5% FBS