SUMMARY OF RESULTS FROM HDF VIABILITY AND ATTACHMENT *IN VITRO*

YYY

BIOE 342

OBJECTIVE

- Viability
  - Estimate viability of Human Dermal Fibroblast (HDF) cells under varying conditions
- Attachment
  - Assess attachment of HDF cells on various surfaces
- Proliferation
  - Assess rate of HDF cell proliferation under different media and different times

PREPARATION FOR VIABILITY TESTS

MTT Viability

- Dilute sample from 50,000 cells/mL to varying concentration
- Seed cells and incubate for 2 days
- Add MTT dye to cells and incubate for 2 hours
- Add Solubilization/Stop solution and incubate for 45 minutes
- Record absorbance at 570 nm

PREPARATION FOR PROLIFERATION TESTS

Anti-PCNA Staining

- Prepare DMEM with 1%, 5%, 10% FBS complete media, blocking buffer, 1^{o} antibody, 2^{o} antibody
- Seed cells in varying complete media and incubate for 2 days
- Add antibodies appropriate for test conditions
- Stain with AEC, hematoxylin, and NH_4OH
- Rinse with DI water and observe

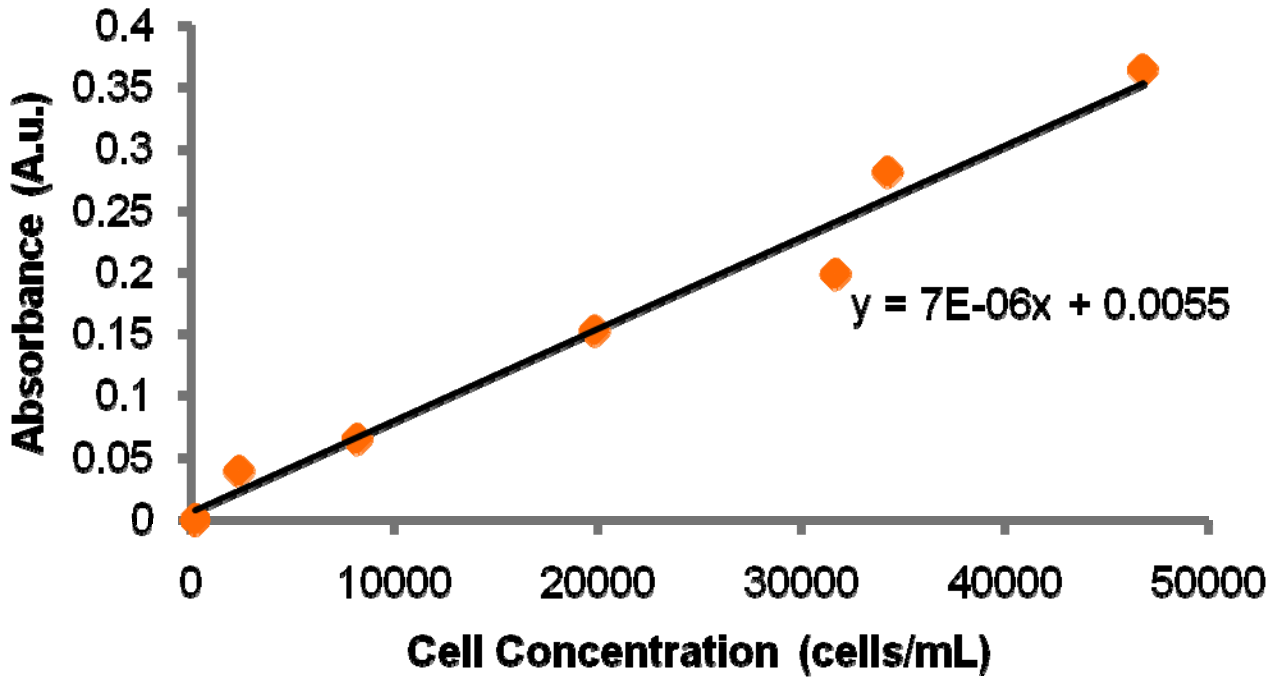
Cell Proliferation Assay

- Dilute cells down to 5,000 cells/mL
- Plate cells in different media conditions (33 wells total) for different day counts (Day 0 – Day 7)
  - A: 1% serum media
  - B: 5% serum media
  - C: 10% serum media
- Coulter count cells each day by removing media, adding PBS, and trypsinizing cells
- Feed cells not harvested

PREPARATION OF QUANTITATIVE ATTACHMENT ASSAY

- Seed diluted (10,000 cells/mL) cells (HDF) in complete media in different conditions:
  - A: Untreated well plates
  - B: Treated well plates
  - C: Treated well plates
  - D: Fibronectin-treated well plates
- Incubate for 30 hours
- Rinse well plates after incubation with PBS
- Count attached cells at varying time intervals:
  - 30 minutes
  - 1 hour 15 minutes
  - 2 hours 30 minutes
  - 4 hours

INCREASE IN CELL CONCENTRATION IS CORRELATED WITH INCREASING ABSORBANCE



The MTT dye reacts with metabolically alive cells. Thus, with different dilutions, we were able to use it to relate cell concentrations and absorbance values

PCNA PROLIFERATION RESULTS

	Cells in S phase (cells/cm^2)	Cells not in S phase (cells/cm^2)	% of cells in S phase
Control A	0	11500	0%
Control B	0	12800	0%
Control C	0	12900	0%
1%	3500	3300	51.5%
5%	7600	6100	55.5%
10%	9800	2200	81.7%

Control A: 200 µL Anti-PCNA 1^o antibody, 200 µL blocking buffer
Control B: 200 µL blocking buffer, 200 µL 2^o antibody
Control C: 200 µL blocking buffer, 200 µL blocking buffer
1%,5%,10%: respective media concentration, 200 µL 1o antibody, 2 µL 2^o antibody

PCNA PROLIFERATION RESULTS DISCUSSED

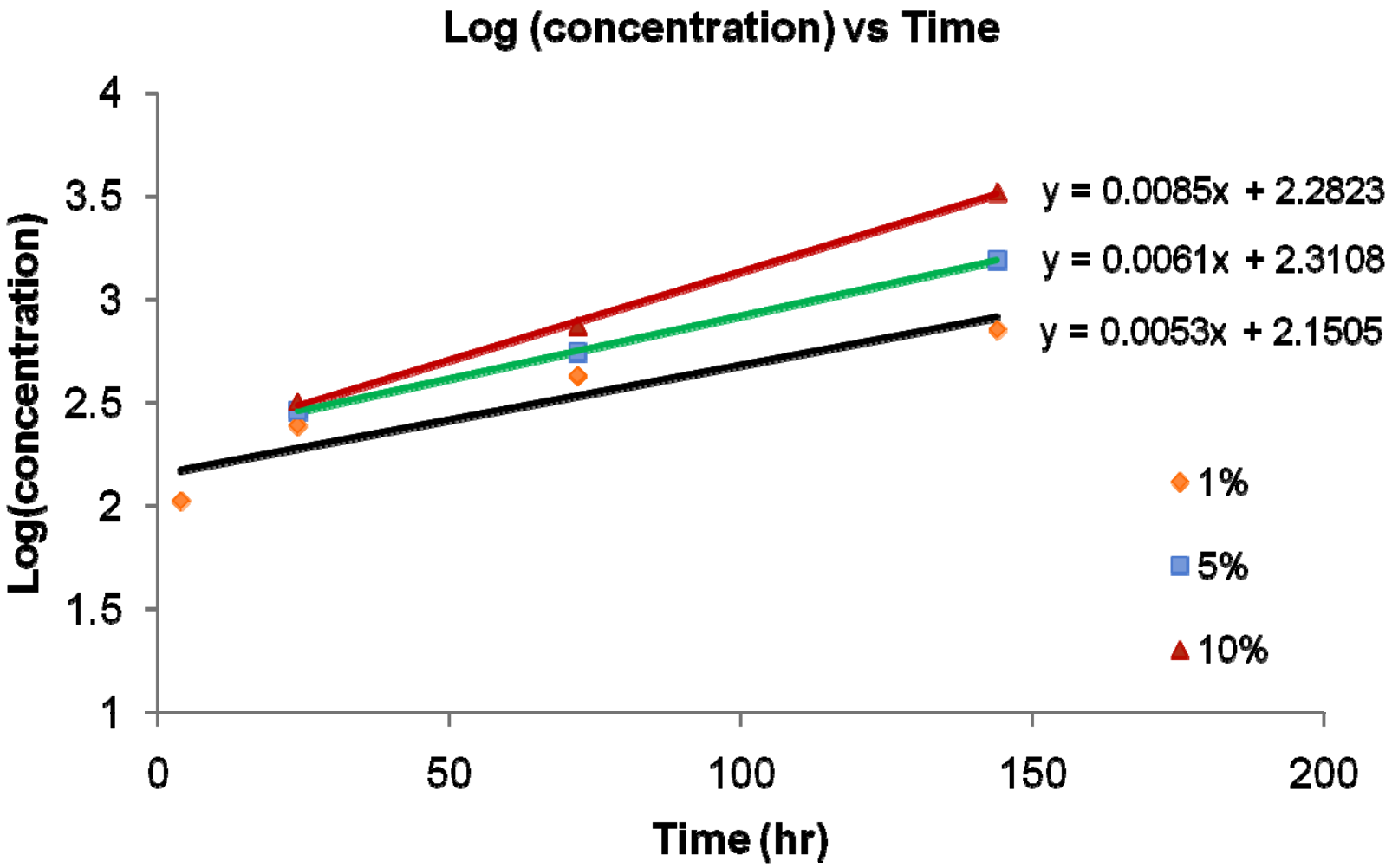
- Higher serum concentration is correlated to high ratio of cells in S phase
- Results suggests higher proliferation rate at higher concentration since more cells in S phase

CELL DENSITY AVERAGE OVER VARIOUS TIME POINTS

	Total Avg cells/0.01 cm^2			
Time (hr)	4	24	72	144
1%	106	245	426	711
5%		288	557	1553
10%		317	746	3330

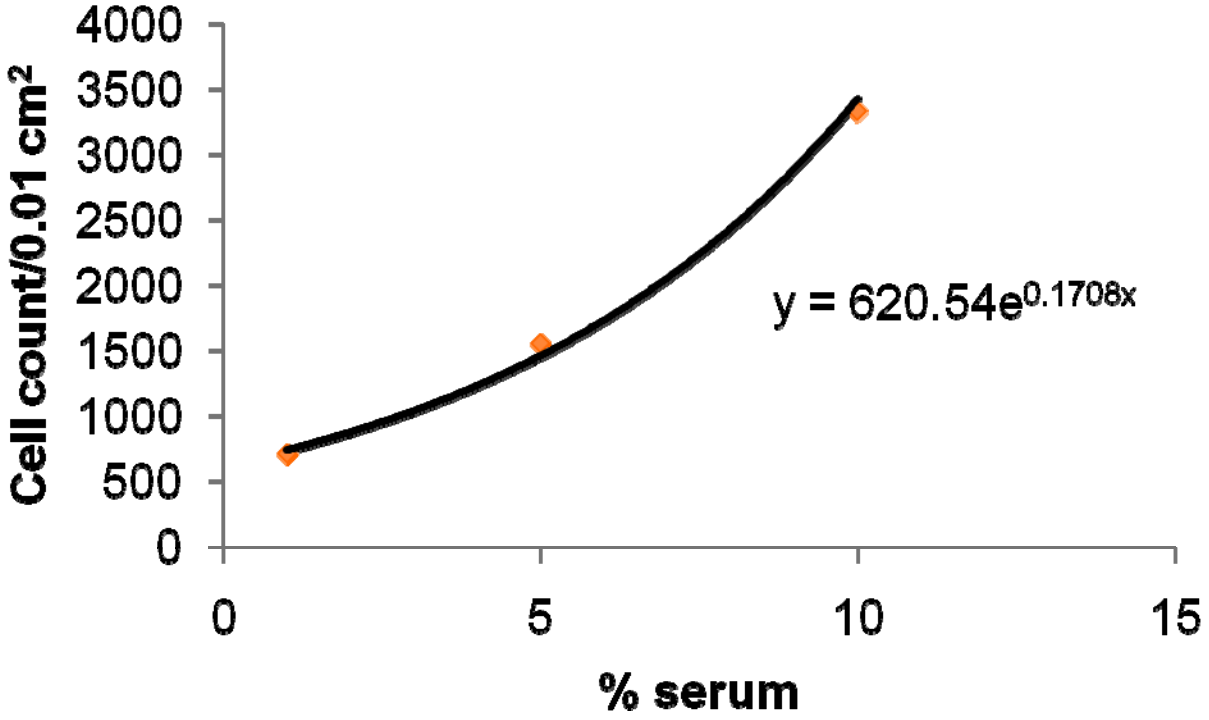
From the cell proliferation data, we can see that the cells are not only proliferating at a quicker rate from day to day but also display increased proliferation rate at increasing concentrations.

Using the ANOVA test, there is a significant difference within the values ($p<0.05$). The Tukey HSD also presented large numbers to verify that the data was statistically significant



Cells are growing exponentially since longer time is associated with a larger concentration.

CELL DENSITY INCREASES WITH SERUM INCREASE



Using an ANOVA test, we obtain a p value that is < 0.05. Using a Tukey HSD, we found that the numbers were all greater than their respective HSD, suggesting a statistically significant result that each value is statistically different.

QUANTITATIVE ATTACHMENT ASSAY RESULTS

Time/Tx	A				B			
Cell density (cells/cm)	1	2	3	Avg	1	2	3	Avg
30 min	200	300	400	300	800	600	1300	900
1 hr 15 min	700	600	500	600	3700	3400	2200	3100
2 hr 30 min	23500	7000	9100	13200	15100	10400	8100	11200
4 hr	13900	20400	28300	20867	34500	22100	26400	27667

The untreated cell plate attachment had significantly different results using an ANOVA test and verified by the Tukey. This suggests that the treated plates are able to have a better cell attachment than untreated plates.

QUANTITATIVE ATTACHMENT ASSAY RESULTS PT 2

Time/Tx	C				D			
Cell density	1	2	3	Avg	1	2	3	Avg
30 min	400	200	200	267	1300	400	400	700
1 hr 15 min	1000	1800	1400	1400	2000	2500	1700	2067
2 hr 30	13100	31500	30900	25167	20000	33700	30900	28200
4 hr	26600	27100	24100	25933	28600	32400	14000	25000

Results obtained from XXX. Test conditions C and D do not show a significant difference in an ANOVA test ($P>0.05$). There seem to be no statistical differences in cell attachment between the two results despite Fn-treated plates showing slight improvement over the TC-treated well plates in data taken at 30 minutes, 1.25 hours, and 2.5 hours

CORRELATION BETWEEN PCNA STAINING AND CELL PROLIFERATION

- The proliferation study correlated well with the PCNA staining assay. Since we realize that a higher concentration of serum in the media yields a higher concentration of cells in the S phase, we could also map this to the results of the proliferation study. The higher concentration of cells in the S phase may have played a significant role in the faster rate of proliferation in cells grown in media with higher concentration of serum.

CONCLUSION AND FUTURE STUDIES

- We can assume from results obtained from the proliferation assay and the quantitative attachment assay that cells grown in TC-treated wells and media with10% serum will have the highest cell count over a week.
- The cell proliferation on different surfaces and concentrations can be monitored using an MTT assay
- More work may be done to determine whether there is a significant difference for cell growth using TC-treated or Fn-treated well plates