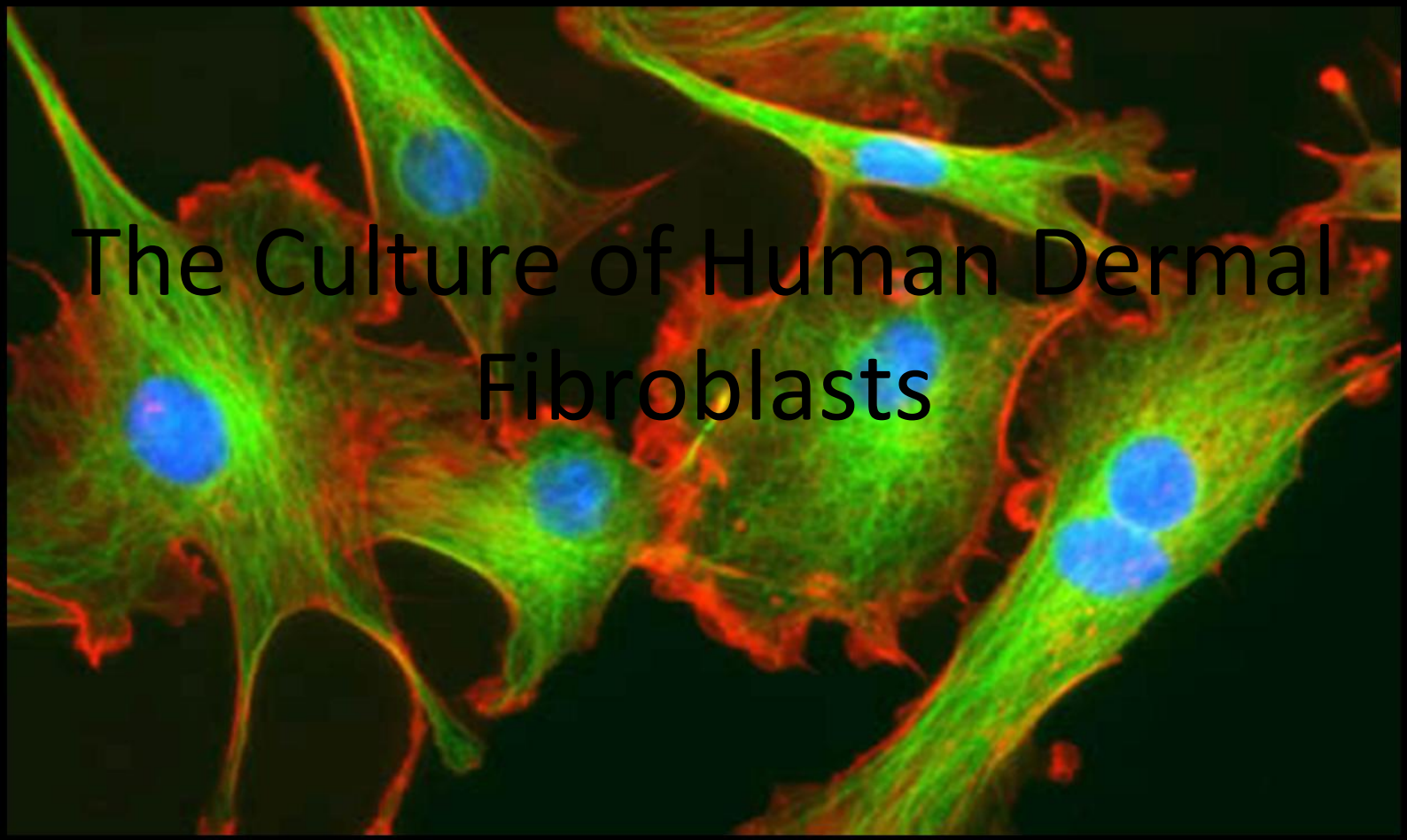
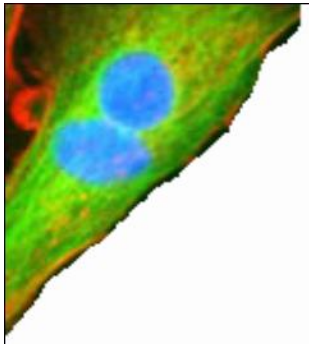
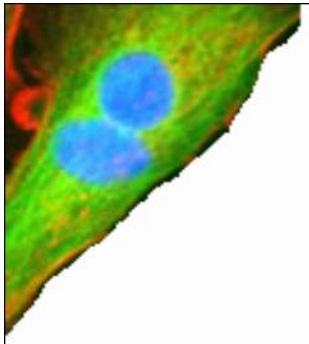
The Culture of Human Dermal Fibroblasts





Objectives

- Qualitatively and quantitatively assess the proliferation rate of HDF cells based on the amount of FBS contained in the media
- Qualitatively and quantitatively the relationship between cell concentration and viability and the response of HDF cells to cytotoxic agents



Cell Proliferation Assays

- Anti-PCNA Assay

  - 20k cells were seeded into 1 mL media (DMEM) @ FBS concentrations of 1%, 5%, and 10% for 48 hours.

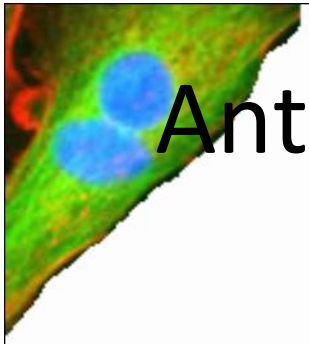
  -Cells were stained w/ Anti-PCNA antibody, which stains nuclei in S-phase red.

  -Hematoxylin was added as a control to stain all cells blue

- The Cell Proliferation Assay

  -5k cells were seeded in 1mL 1, 5, & 10 % FBS DMEM.

  -Cells were trypsinized and counted using Coulter counter 4 hrs, 2, 5, & 7 days after seeding.

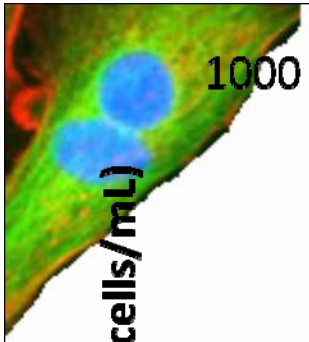


Anti-PCNA staining reveals more cells in S phase in 5 & 10 % FBS

- Cells in all media types displayed similar morphologies but different con--fluencies

	Confluency	Red Nuclei
1% FBS	30-40%	30-40%
5% FBS	60-70%	80-90%
10% FBS	60-70%	80-90%

- The 5 & 10 % FBS cultures had far more cells in S-phase, as indicated by the staining, but no difference was discernible between the two



HDF Cells show logarithmic growth in all types of media

Cell Concentration (Thousands of cells/mL)

1000
100
10
1

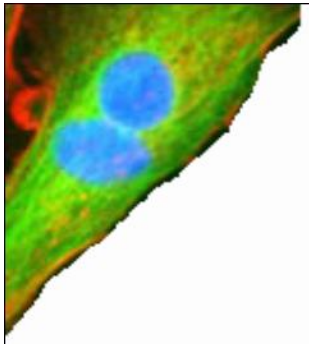
0
2
4
6
8

Days After Seeding

1% FBS
5% FBS
10% FBS

*
*
*

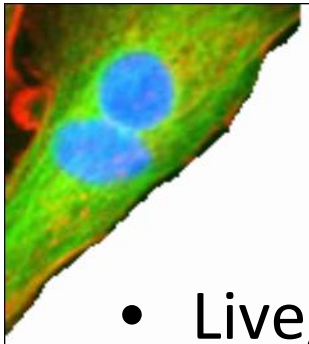
* *Significantly different using single variable ANOVA and Tukey's HSD test w/ p < .01*



Both assays show growth rate higher in media with higher % of FBS

- Anti-PCNA staining indicates more cells in S-phase for 5 & 10% FBS media.
- Cell proliferation assay indicates cells proliferating more rapidly in 5& 10 % media.

  -Doubling time for 1% cells is two times larger than 5 & 10 %
- Can conclude: percent of cells in S-phase at any given time is indicative of their proliferation rate.



Cell Viability Assays

- Live/Dead Assay

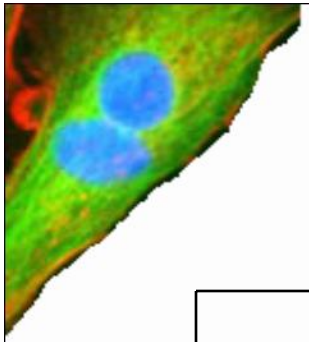
  -15k HDF were plated in 10% FBS media. Before the stain, media was replaced with either EtOH, PBS, or PBS + 2 drops EtOH

  -AM/EthD was added to stain live cells green and the nuclei of dead cells red

- MTT Assay

  -HDF were plated at different concentrations in 10% FBS DMEM. Cells were counted using Coulter Counter. MTT reagent was added and absorbance was recorded, calibrated to vacant control.

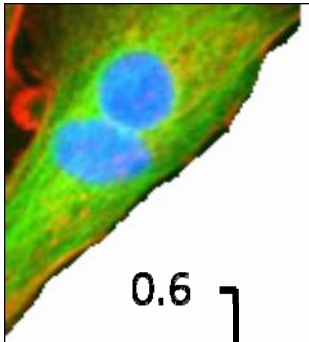
  -MTT reagent's absorbance increases as it is metabolized by living cells, indicating viability.



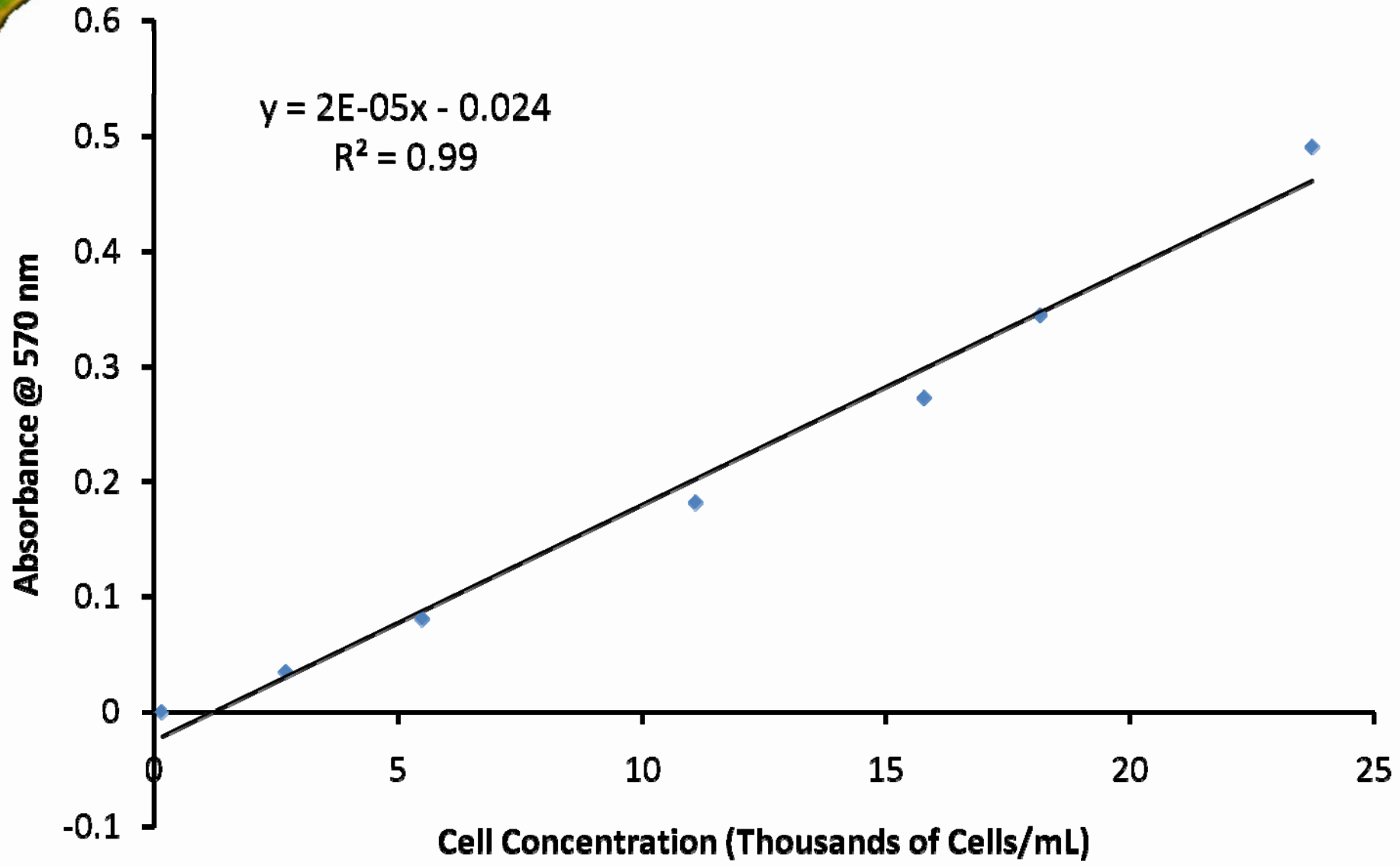
Live Dead Assay

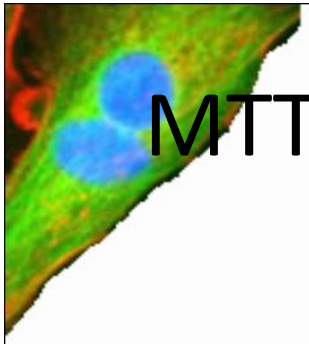
Condition	AM stain (live cells)	EthD (dead cells)
PBS	Nearly all the cells are stained green	Floating cells have red nuclei
EtOH	No green cells	All cells' nuclei stained red
PBS + 2 drops EtOH	All cells green except for small patches	All nuclei in patches red (where drops fell)

- 1st condition indicates that under normal conditions, nearly all cells are viable
- 2nd and 3rd conditions indicate that EtOH kills cells on contact, but is not instantly lethal if dilute



Absorption of MTT increases with Cell Concentration



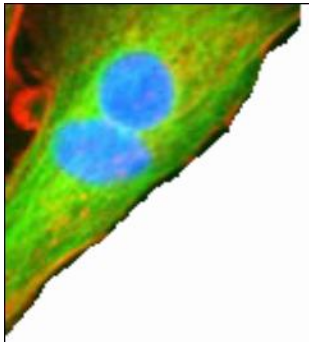


MTT and Live/Dead Assay show most cells viable in normal conditions

- MTT assay shows absorbance linearly proportional to viable cell concentration

- Live/Dead Assay shows most cells are viable under normal conditions, ~ 99%

  -Viability can be greatly decreased by contact with cytotoxic agent



Conclusions

- HDF proliferates more quickly in 10% FBS DMEM as compared to lower concentrations

  -The percent of cells in S phase is indicative of its proliferation rate

- MTT assay has a linear relationship with viable cell concentration.

  -Cells ~100% viable under normal conditions

  -If the results of MTT are lower than expected, viability is low