Viability and Proliferation in HDF Cells

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Bioe 342

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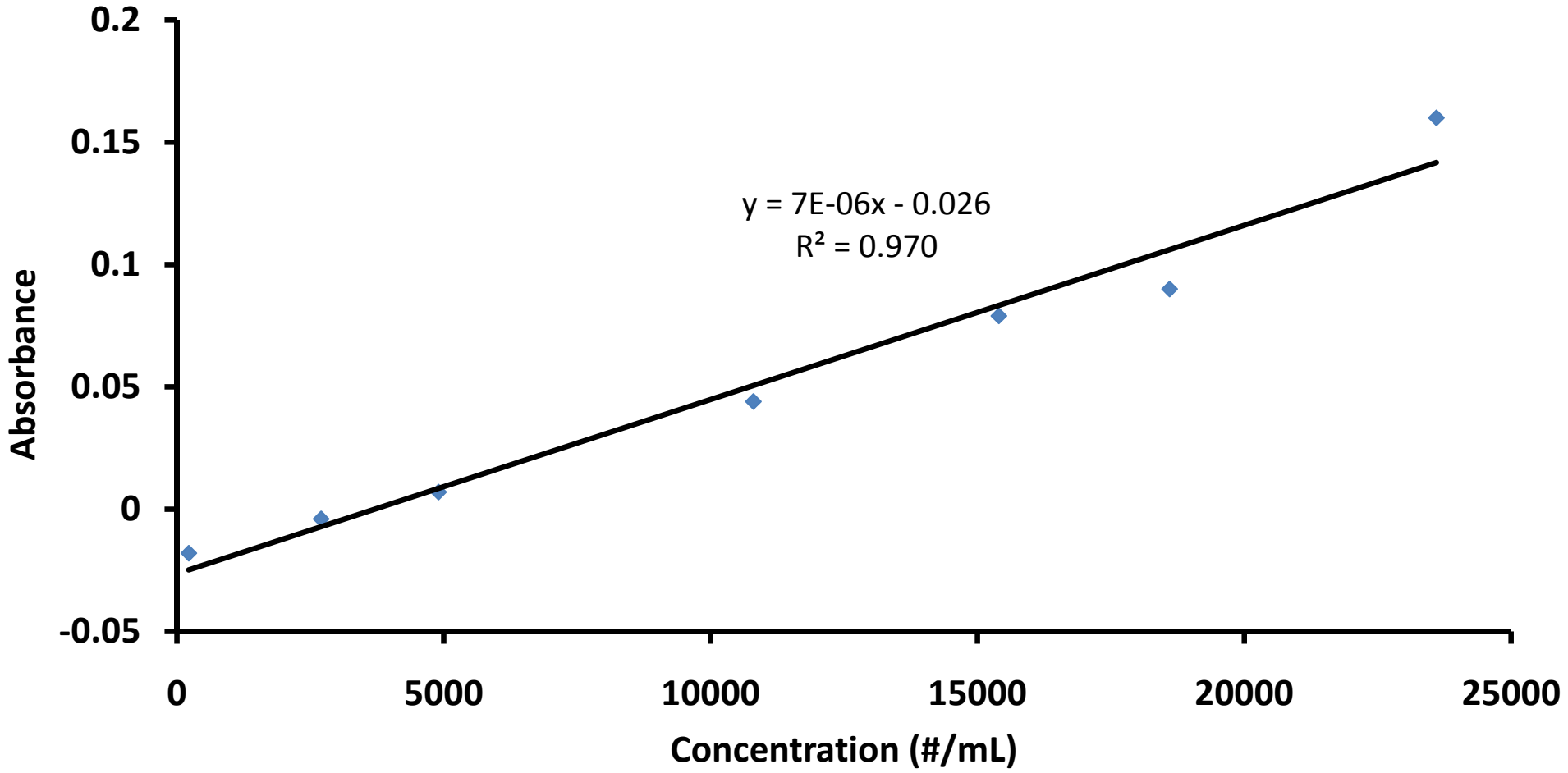
Objectives

- Formulate a relationship between the absorbance of a MTT viability test and cell number.
- Asses the impact of ethanol on living cells using the Live/Dead Fluorescence assay.
- Conduct a cell proliferation assay to asses the effect of serum on the growth and replication of HDF cells.

Methods- MTT Viability Test

- HDF P5 cells were seeded on 2 sets of 7 cells of 24-well plate in concentrations of stock(50,000 cells/mL), 1:1.5, 1:2, 1:3, 1:6, 1:12, and control (no cells)
- After 2 day period, MTT dye was added to each well on MTT plate
- The absorbance of the cells from the MTT plate was measured by a spectrophotometer.
- The other plate of cells was trypsinized and each well was counted on the Coulter Counter.

Linear Relationship between Absorbance and Cell Count



- Relationship between absorbance and cell count linear (R=0.97)

Methods- Live/Dead Fluorescence

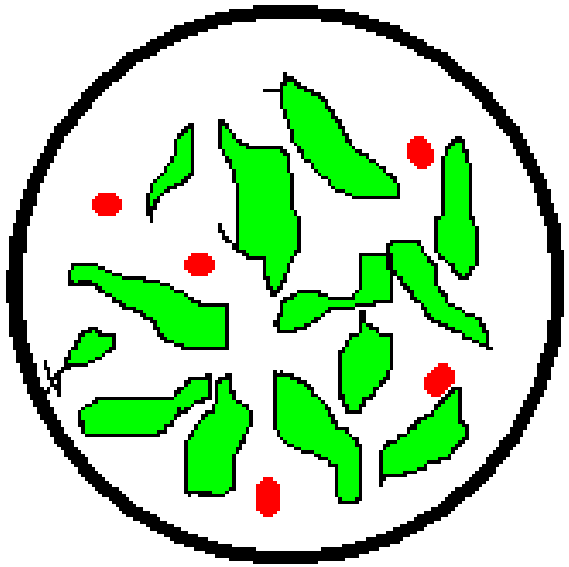
- HDF P5 cells were seeded in 9 wells of a 24-well plate
- After a 2 day period, the wells were separated by groups of three into the three test conditions: 250 µL PBS, 250 µL ethanol, and 250 µL PBS and 2 drops ethanol
- 100 µL Cacien AM and Ethidium Homodimer mix was added to dye the cells
- Cells were viewed under a fluorescent microscope and observations recorded

Live/Dead Fluorescence Assay Observations

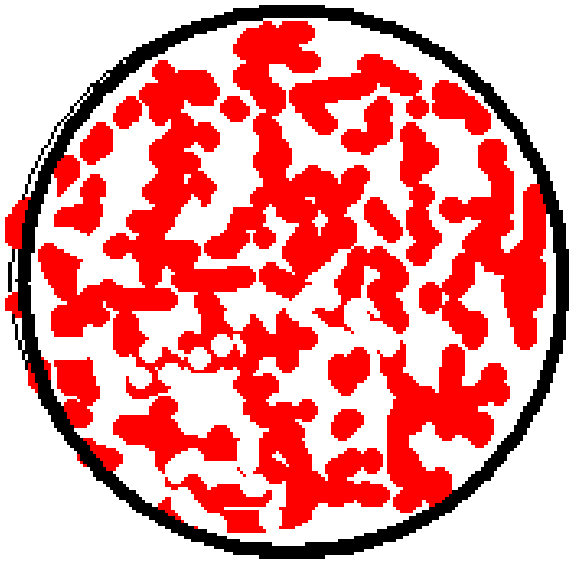
Test Condition	Observations
250 µL PBS	Over 95% of cells appear green Very few red cells randomly dispersed
250 µL ethanol	100% of cells appear red
250 µL PBS and 2 drops ethanol	Both red and green cells Red cells appear in circular spots surrounded by green cells Few red cells dispersed with green

Appearance of Live/Dead Assay

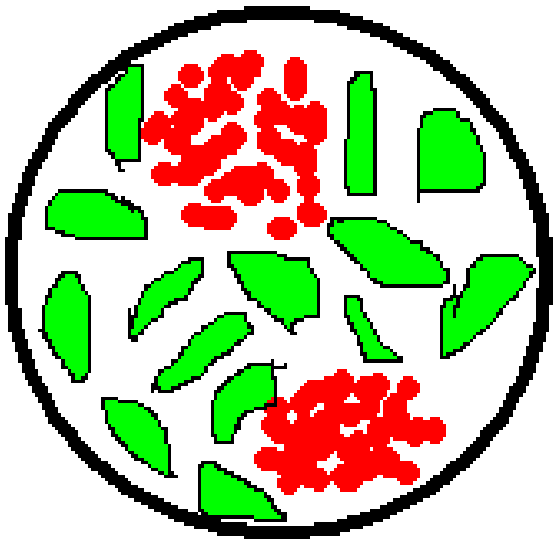
PBS



Ethanol



PBS and Ethanol



- Live cells appeared green
- The nuclear material of dead cells appeared red
- Ethanol kills all cells it contacts hence all red in condition 2 and spots of red in condition 3

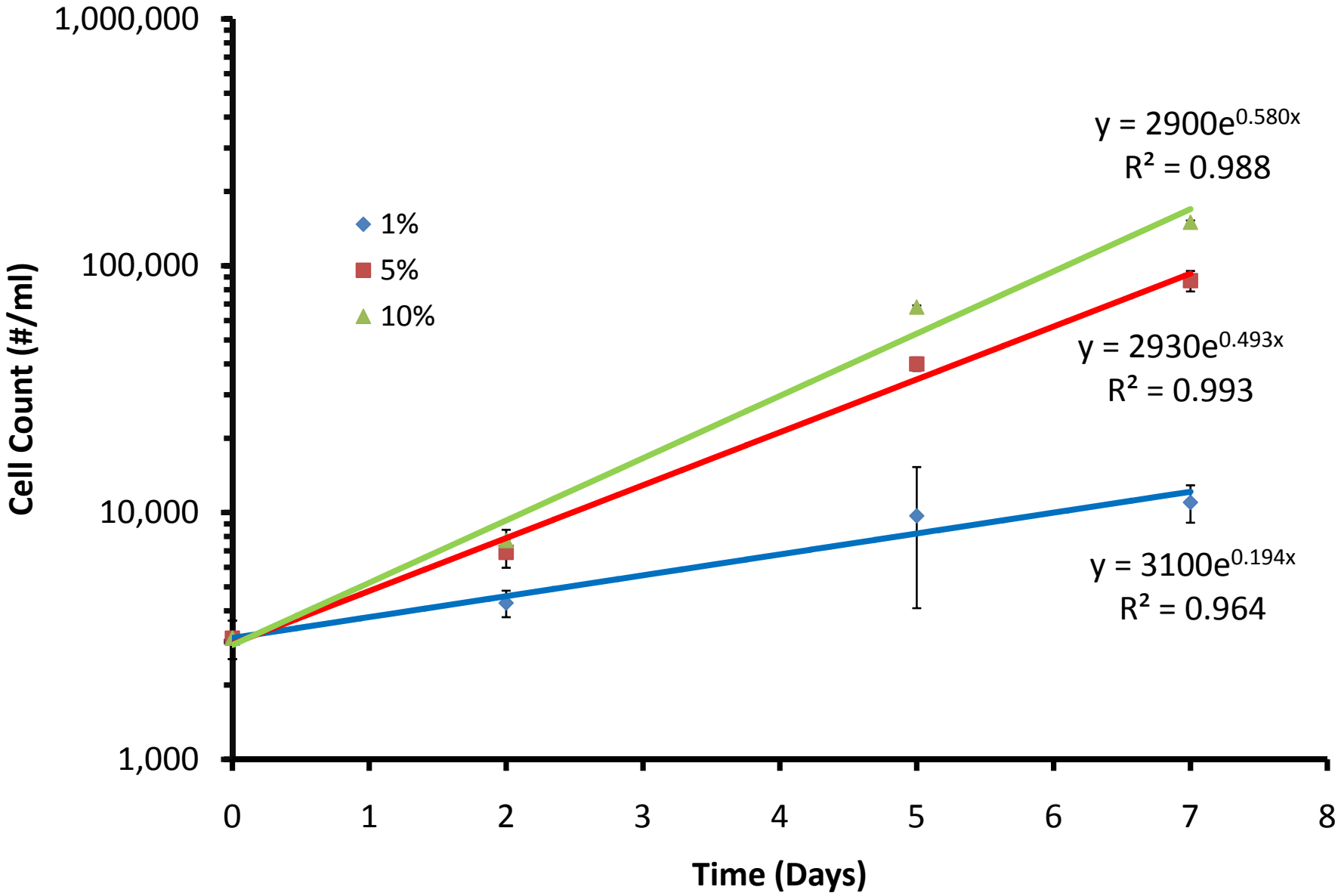
Assessing Cell Viability Using MTT and Live/Dead Assays

- Coulter Counter counts overall number of particles not number of live cells
- MTT results therefore not entirely accurate, since the absorbance is only from live cells
- A more appropriate relationship between absorbance and cell viability determined by:
  - Perform MTT and find absorbance for 1st set of conditions
  - Perform Live/Dead Fluorescence Assay on 2nd set of test conditions
  - Count cell concentration of live cells in Live/Dead assay using the hemocytometer
  - Find relationship between Live/Dead concentration and absorbance

Methods-Cell Proliferation

- HDF P6 cells were seeded at a concentration of 5000 cells/mL onto a total of 33 wells over 2, 24-well plates
- All cells were seeded with media with 1% Fetal Bovine serum (FBS) and allowed to incubate for 4 hours
- After the 4 hours, the cell concentrations of 6 wells were counted using the coulter counter
- The other wells were split into 3 test conditions of 1%, 5%, and 10% FBS in media
- 3 wells of each test condition were counted using the coulter counter on days 2, 5, and 7

Cell Proliferation increases with % FBS



Cell Proliferation Analysis

- Cell concentration was statistically significant in the 10% FBS test group between days 2, 5, and 7 with $P<<0.05$
- Cell count on day 7 was statistically significantly greater with 10% FBS ($P<<0.05$)
- Growth for 1%, 5%, and 10% FBS was exponential ($R>0.95$)

Summary

- MTT assay established relationship between absorbance and cell concentration such that:
- Live/Dead Fluorescence assay allowed for distinguishing live and dead cells with fluorescent microscope.
- If used in conjunction Live/Dead and MTT could establish a more accurate relationship between absorbance and cell viability
- Cell proliferation was greatest with 10% FBS and decreased with decreased FBS percent.