Tissue Culture Conditions Affect Cell Viability & Proliferation

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Objectives

- To analyze & develop a relationship between viable cells & absorbance.
- To evaluate the effect of ethanol on live, HDF cells.
- To observe & quantify the effect of various serum conditions on HDF cell proliferation.

MTT Viability Assay Methods

- Seeded 6 samples of HDF cells at various dilutions & one control well with complete media.
- MTT Dye Cells:
  - Incubated cells with MTT Dye & then with Solubilization/Stop Solution.
  - Read cell absorbance with Spectrophotometer at 570 nm.
- Coulter Counter Cells:
  - Washed & trypsinized cells.
  - Transferred cells into isoton vials to determine cell counts with a Coulter Counter.

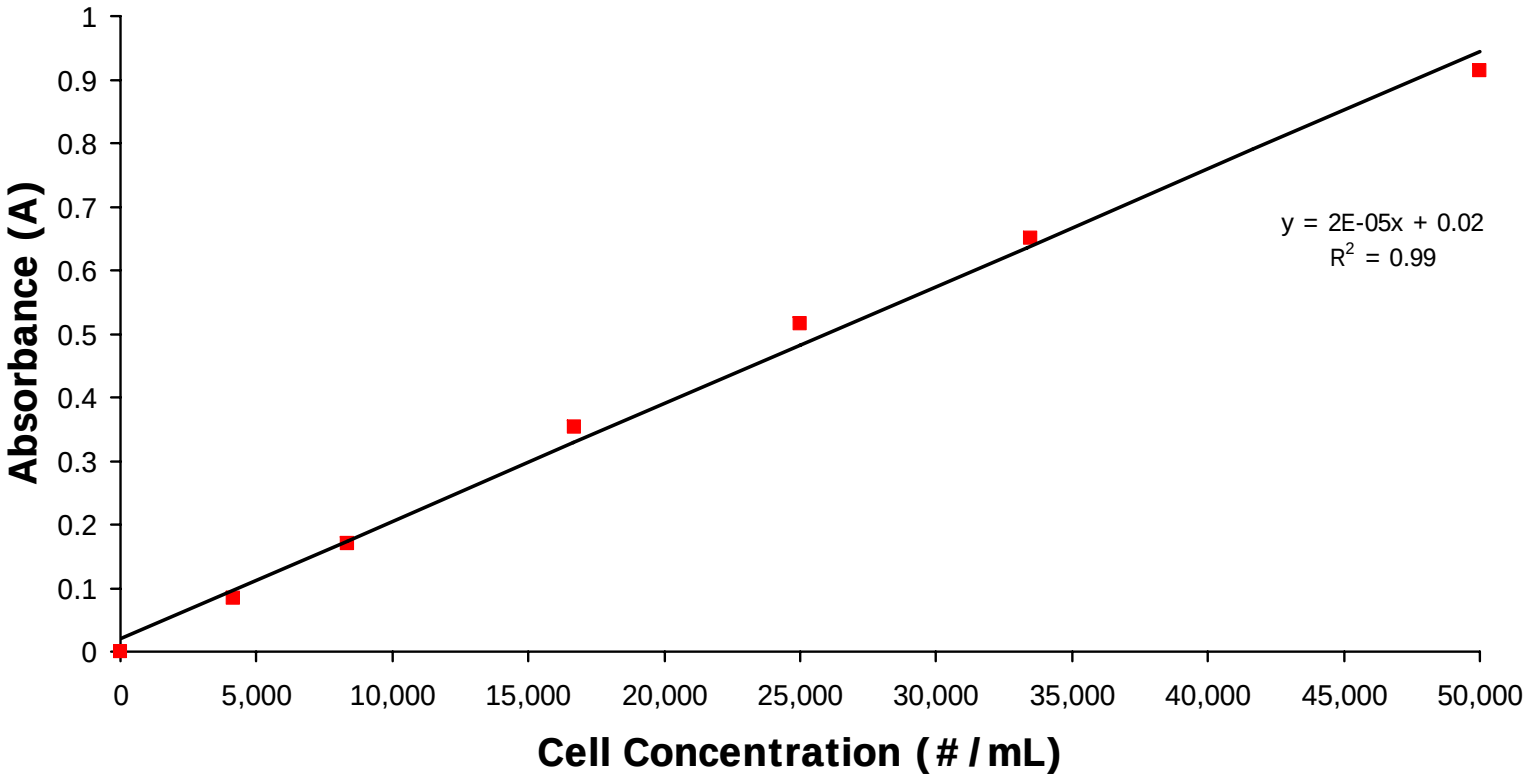
Live/Dead Fluorescence Assay Methods

- Seeded HDF cells on a TC-treated plate.
- Subjected cells to three test conditions:
  - PBS + Dye
  - Ethanol + Dye
  - PBS + 2 Drops Ethanol + Dye
- Incubated cells & then observed them under a fluorescence microscope.
- Live cells stain green & dead cells stain red.

Cell Proliferation Assay Methods

- Seeded & incubated cells on TC-treated plates.
- Day 0 cells grown in 1% FBS DMEM.
- Day 1, 3, & 6 cells grown in 1, 5, or 10% FBS DMEM.
- Observed cells with a light microscope.
- Trypsinized cells on respective days & transferred them to isoton vials.
- Obtained cell counts with a Coulter Counter.

Absorbance & Cell Concentration are Linearly Related

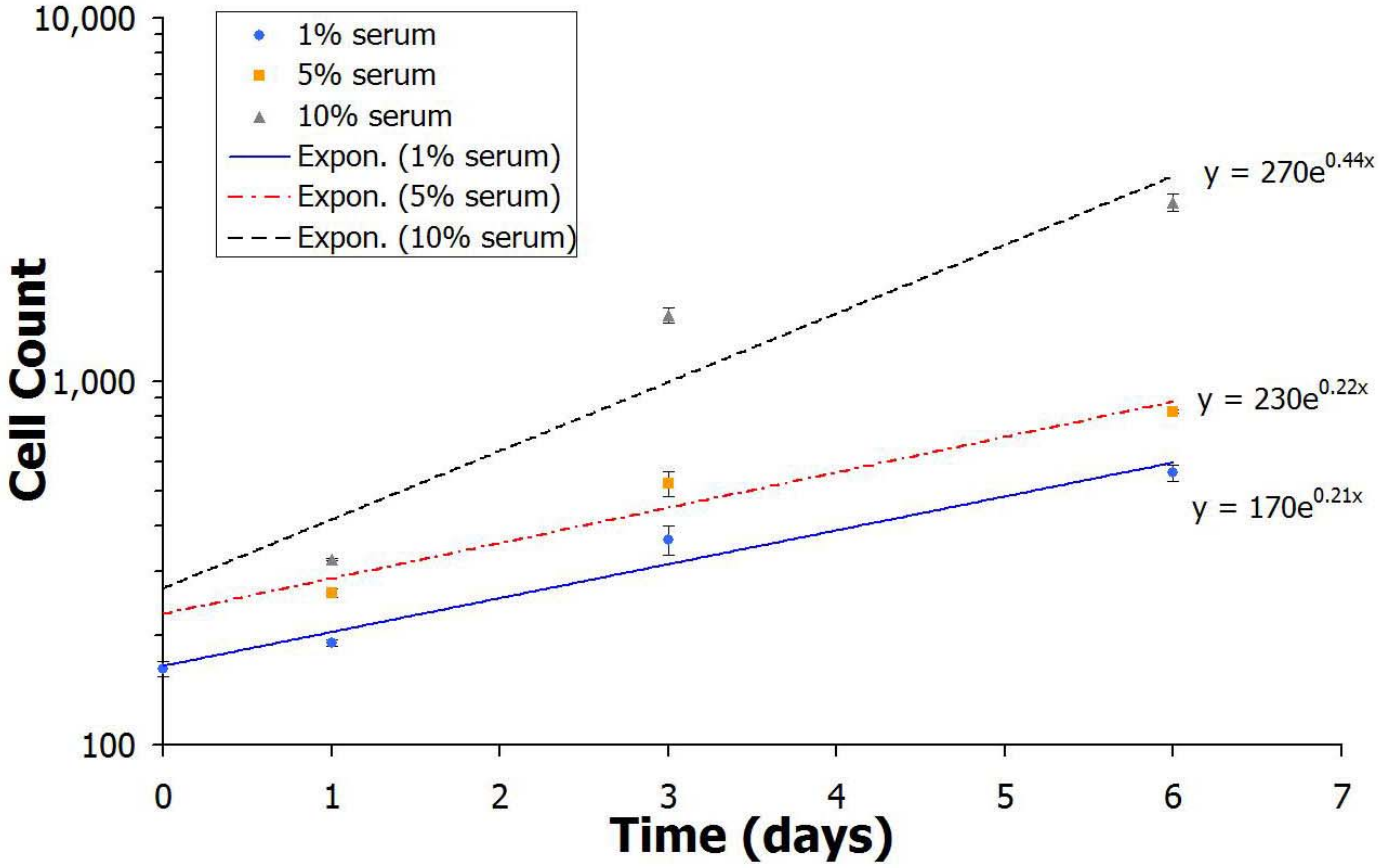


- Higher cell concentrations result in increased absorbance

Ethanol is Toxic to HDF Cells

Condition	Observations	Toxicity
PBS + Dye	All cells stain green. Cells are spread out & attached with pseudopodia extended.	PBS is not toxic to cells.
Ethanol + Dye	All cells stain red. Cells are attached. No visible pseudopodia extended.	Ethanol is toxic to cells.
PBS + 2 Drops Ethanol + Dye	Cells in center of well stain red; surrounding cells stain green. 50% of cells are attached; 50% unattached. Some green cells appear to be balled up.	Lower toxicity of ethanol when its concentration is decreased.

Higher FBS Concentrations Promote Cell Growth



- Data taken from XXXX

FBS Concentrations affect Proliferation

- Cell growth is exponential.
- Cell numbers at Days 1, 3, & 6 are statistically different from one another. ($p<0.001$)
- Day 6 cells grown in 1, 5, or 10% serum are statistically different from one another.
  - $p<0.05$ for 1% vs. 10% & 5% vs. 10% serum
  - Anova & Tukey Test used for statistics.
- Population doubling time:
  - Calculation: ln(2) / slope of fit
  - Decreases with increased % serum

% FBS	Doubling Time (days)
1	3.20
5	3.10
10	1.60

Assessment of Cell Viability Assays

- Both assays measure the amount of live, viable cells.
- MTT Viability Assay:
  - Assesses viability indirectly.
    - Coulter counter counts cells to determine concentration.
    - Absorbance is dependent on cell concentration.
    - Dead cells exhibit no absorbance.
  - Disadvantage: Coulter Counter counts dead cells.
- Live/Dead Fluorescence Assay:
  - Assesses viability directly.
    - Staining dye allows observance of the fraction of live & dead cells with a fluorescence microscope.
  - Disadvantage: No precise cell counts collected; only qualitative data.

Conclusions

- Absorbance and cell concentration are linearly related to each other.
- Ethanol is toxic to HDF cells.
  - Cell nuclei stained red in live/dead assay.
- PBS is nontoxic to HDF cells.
  - Cells stained green in live/dead assay.
- FBS promotes cell proliferation.
  - Exponential cell growth was observed.
  - Doubling time decreases as % serum increases.
    - Serum contains growth factors & other nutrients.