Use of Cellular Processes to Gather Data for a Cell Culture

BIOE 342

Objectives Achieved by Observation of Cellular Processes

- To measure cell concentration based on absorbance of light
  - MTT Viability Test
- To determine the effects of a toxic reagent on cells
  - Live/Dead Fluorescence Assay
- To assess attachment of HDF cells to different surfaces
  - Quantitative Cell Attachment Assay

Using Absorbance to Measure Cell Concentration

- Seeded sample cell concentrations two sets in 24 well plates
- Cells incubated 2 days
- Relationship between absorbance and concentration developed:
  - One set of test conditions counted with a Coulter Counter to determine cellular concentration
  - Second set of test conditions treated with MTT dye, absorbance measured with a Spectrophotometer

Dilution	Concentration
Stock	50,000 cells/mL
1:1.5	33,500 cells/mL
1:2	25,000 cells/mL
1:3	16,700 cells/mL
1:6	8,330 cells/mL
1:12	4,170 cells/mL
Control	0 cells/mL

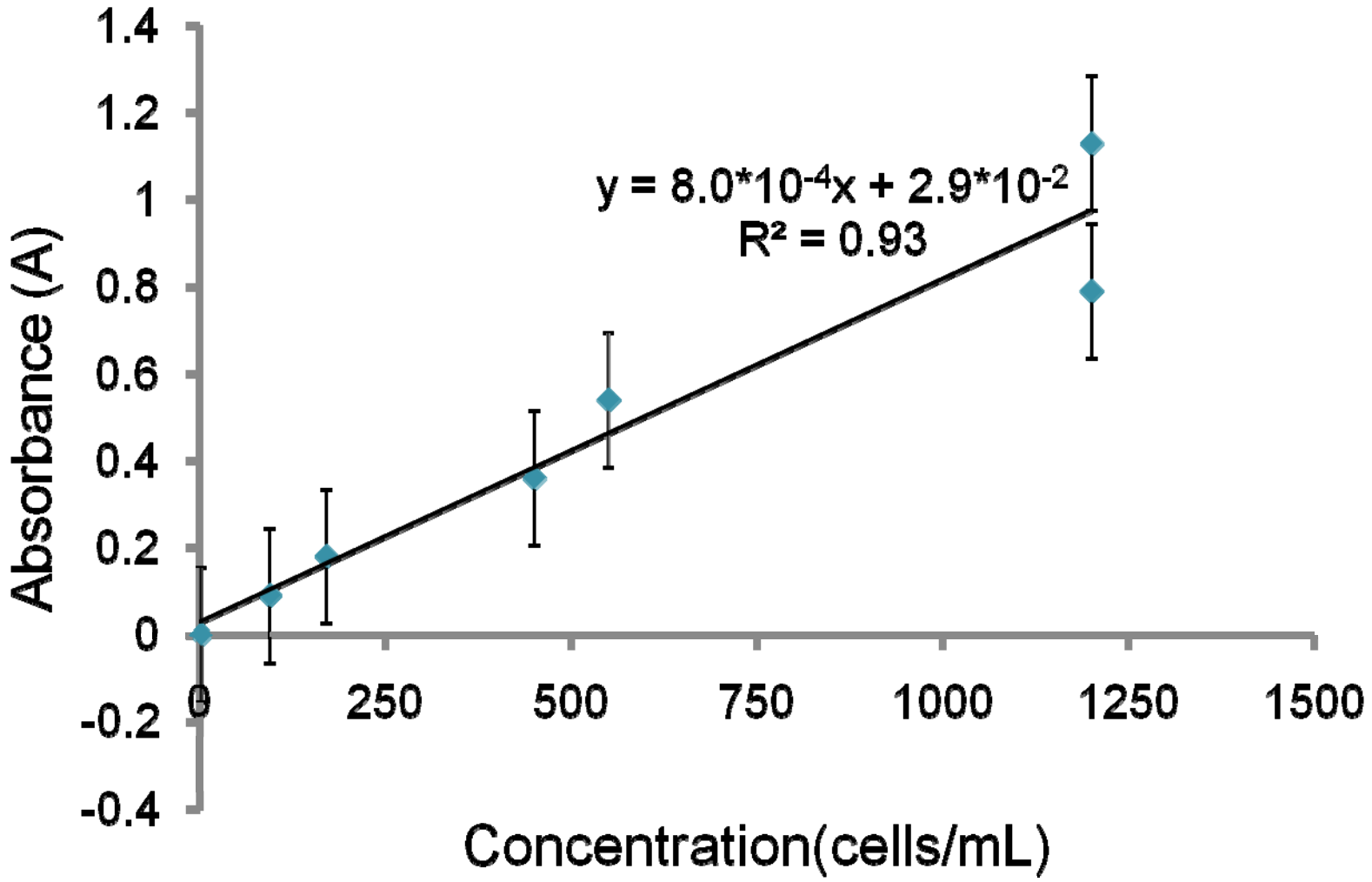
Differentiating Between Live and Dead Cells

- Seeded cells in a 24 well plate and incubated 2 nights
- Three different experimental treatments were applied:
  - 250µL PBS, 100µL dye (EthD-1, Calcein AM)
  - 250µL Ethanol, 100µL dye
  - 250µL PBS, 2 drops Ethanol, 100µL dye
- Observed cells under a fluorescent microscope.

Assessing Attachment to Different Surfaces

- Seeded Cells in TC-treated polystyrene, Fn-coated polystyrene, and untreated polystyrene 24 well plates
- Incubated cells and counted using a light microscope at 30 min, 1 hr 15 min, 2 hr 30 min, 4 hr after seeding

Absorption and Concentration are Linearly Related



Absorbance Caused by Cell Metabolic Activity

- Cell concentration based on mean of cell numbers with n=3
- Absorbance at 570nm increases as concentration of cells increases
  - Metabolic activity of cells results in MTT dye becoming a purple color
  - More cells = more metabolic activity over a given period of time

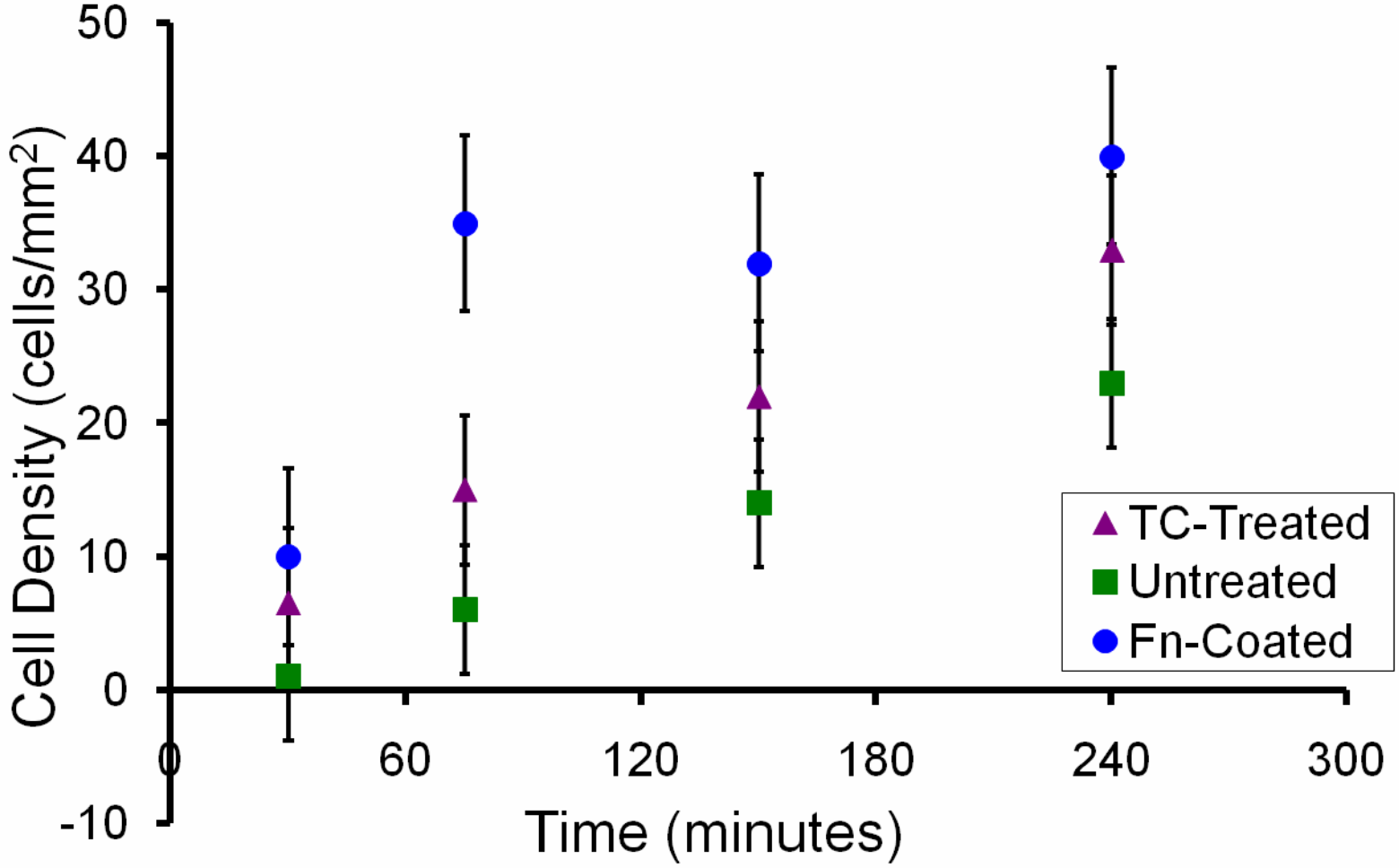
HDF Cells are Killed by Ethanol, Unharmed by PBS

Treatment	Observations
250µL PBS	-Green dots cover wells evenly -Almost no Red dots observed
250µL Ethanol	-No Green dots observed -Red dots visible covering wells evenly
250µL PBS, 2 drops Ethanol	-Green dots observed in patches -Red dots observed in areas with no Green dots

Coloration due to Fluorescent Dyeing of Nucleus

- Green dots are stained nuclei of live cell
- Red dots are stained nuclei of dead cells
- Light microscopy after treatment showed no change in morphology or confluency
- Observations based on n=3 per treatment

Fn-Treated Well Plates Have the Most Cells Adhered at a Given Time*



*TC-Treated data is an average with Vani Rajendran's data; data for Untreated plates collected by Vani Rajendran

Fn-Coated Plates Have a Statistically Significant Advantage

- At each time point, n=3 samples per condition observed
- Fn-Coated well plates have statistically significantly higher numbers of cells attached at a given time (one-tailed t-Test, $p<.01$)
- TC-treated plates have an advantage over untreated plates
  - Statistically significantly higher numbers of cells attached at a given time to TC-treated plates (one-tailed t-Test, $p<.01$)

Metabolic or Fluorescent Assays Supplement Light Microscopy

- Cells can be well attached to an appropriately treated surface within hours
  - 80% of cells seeded attached to Fn-coated plate within 4hrs
  - Majority of cells look elongated and growth organized in light microscope, few balled-up cells
- Toxic substances kill cells without noticeable effect
  - After H_2O_2 treatment, morphology and confluency did not change
- Metabolic assay or fluorescence staining can detect the drop in number of viable cells
  - Dead cells will stain red, or will not affect MTT dye through metabolic activity

Implications of Data Gathered from Observing Cellular Processes

- MTT Viability Assay relies on Cellular Metabolism
- Cell Death Caused by Exposure to Toxic Materials showed effective use of fluorescence assay
- Time necessary for cells to attach varied between surface treatments, and showed which was better