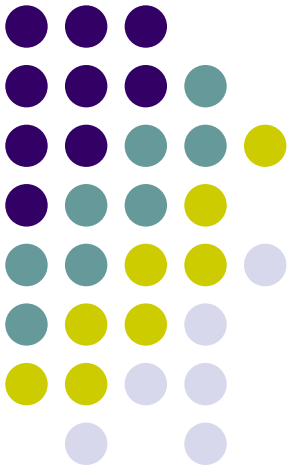
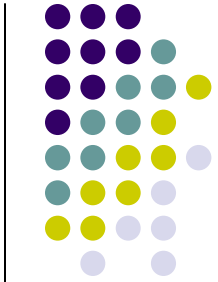
Viability and Attachment Characteristics of Human Dermal Fibroblasts (HDFs) *in vitro*

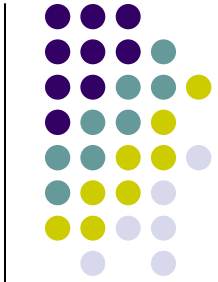


Objectives



- To establish a linear relationship between absorbance and living HDF concentration
- To investigate HDF survival after ethanol application
- To analyze surface attachment characteristics of HDFs to TC treated and Fibronectin (Fn) coated plates

MTT Assay Methods



- Seeded 0.5 mL of samples 1-7 in 10% FBS + complete media on 2 separate TC plates
- Incubated plates for 2 days
- Applied MTT dye to Plate 1 and incubated for 2 hrs
  - Added stop solution for 45 min
- Coulter count Plate 2 to determine cell concentrations
- Recorded absorbance of Plate 1 wells
  - Used spectrophotometer @ 570nm
  - Sample 7 acted as blank

Sample	Cell Concentration (cells/mL)
1	50,000
2	33,500
3	25,000
4	16,700
5	8,330
6	4,170
7	0 (control of media)

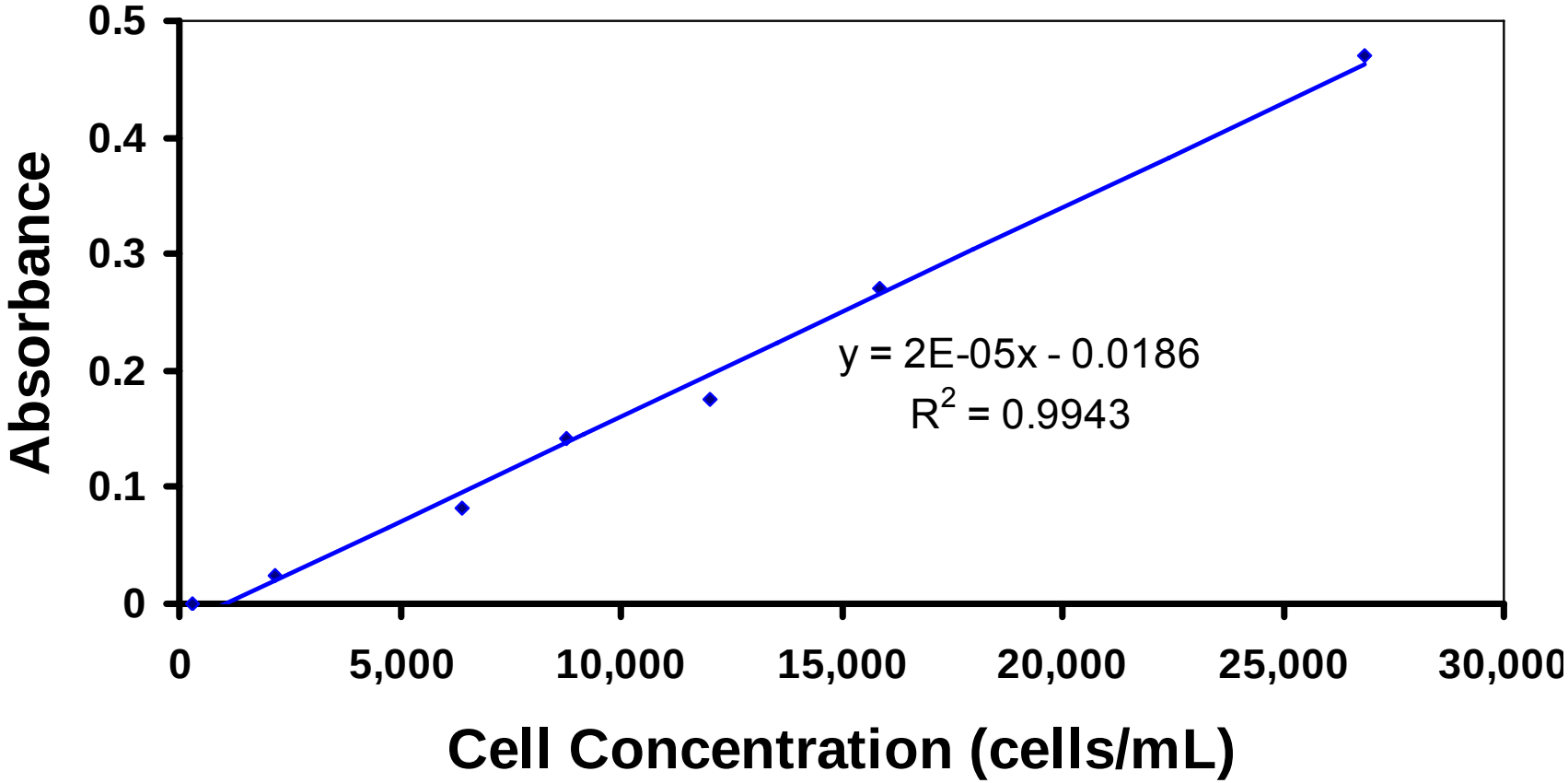
Live/Dead Fluorescence Assay Methods

- Seeded 9 wells with 1 mL cell suspension in TC plate
  - ~50,000 cells/mL in cell suspension
  - Cells exist within 10% FBS and complete media
- Incubated plate for 48 hrs
- Added 100 μL of Live/Dead dye to 9 wells
  - Condition A: Add 250 μL of PBS to wells 1-3
  - Condition B: Add 250 μL of ethanol to wells 4-6
  - Condition C: Add 250 μL of PBS + 10 μL ethanol to wells 7-9
- Incubated cells with Live/Dead dye for 30 min at ~75°F
- Observed cells under light microscope at 100x mag.
- Observed cells under Nikon fluorescent microscope

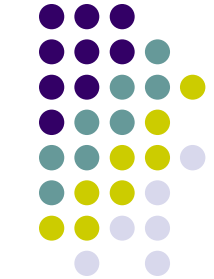
Quantitative Cell Attachment Assay Methods

- Seeded 1 mL of cell suspension on one TC treated and one Fn coated polystyrene plate
  - ~50,000 cells/mL in cell suspension
  - Cells in 10% FBS and complete media
- Measured cell attachment at 0.5, 1.25, 2.5 and 4 hour incubation times
- For each time point:
  - Each well was rinsed 3 times with PBS
  - Counted attached cells in 0.01 cm^2 grid at 100x magnification with light microscopy
- Noted morphology changes

Live Cell Concentration and Absorbance Exhibit Linear Relationship



Live & Dead Cells are Indistinguishable Under Light Microscopy for Cond. A-C

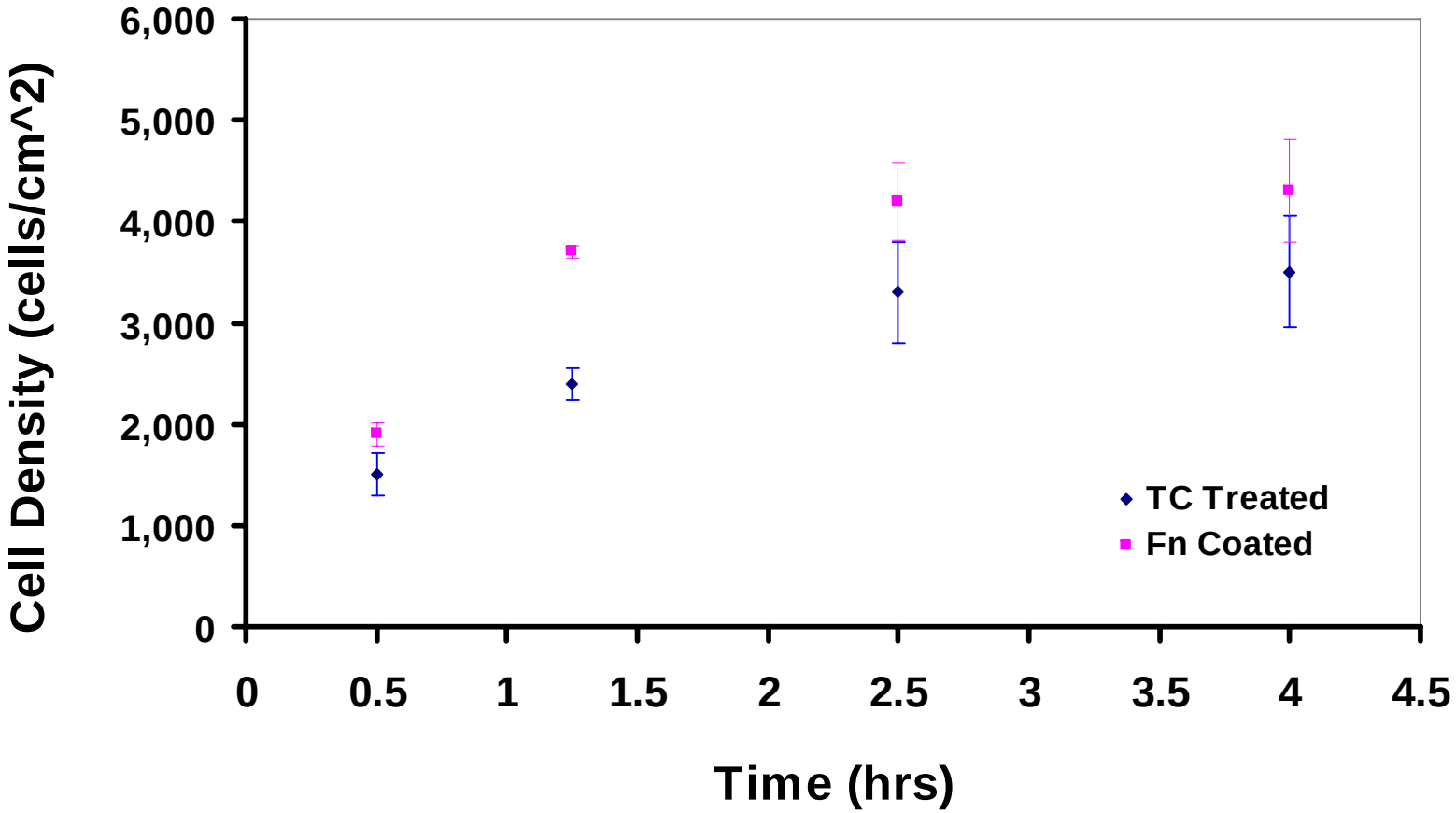


Condition	Confluency	Morphology	Color
A (PBS)	70-80%	Cells have extended pseudopodia and exhibit elongation	Colorless
B (Ethanol)	70-80%	Similar observations of Condition A	Colorless
C (PBS + 10 μL Ethanol)	70-80%	Similar observations of Condition A & B	Colorless

Fluoroscopy Distinguishes Live and Dead Cells Based on Cytoplasm & Nuclei Color

Condition	Morphology	Color
A	Same under light microscopy	• Nearly 100% cells have dyed green cytoplasm • Nuclei are unstained except for 8 spherical cells w/ red dye
B	Same under light microscopy	• No cells have dyed green cytoplasm • 100% of cell nuclei are stained red
C	Same under light microscopy	• 70% of cytoplasm have dyed green cytoplasm • 30% of cells exhibit red nuclei

HDFs Exhibit Different Attachment Characteristics Depending on the Surface



HDFs Extend Pseudopodia and Spread Faster on the Fn Coated Plate

Time (hrs)	TC Plate Observations	Fn Plate Observations
0.5	• Cells are small & spherical • No pseudopodia extension	• Cells are small & spherical • No pseudopodia extension
1.25	• 60% of cells are spherical • 40% of cells are extending pseudopodia	• 40% of cells are spherical • 60% of cells are extending pseudopodia
2	• 100% of cells have lost their round shape and exhibit slight spreading	• 80% of cells are irregularly shaped with clear spreading • 20% of cells have elongated disk shape
4	• 50% of cells have elongated disk shape • 50% of cells are irregularly shaped with clear spreading	• 70% of cells have elongated disk shape • 30% of cells are irregularly shaped with clear spreading

Two Tailed T Test Indicates Non-statistically Significant Differences

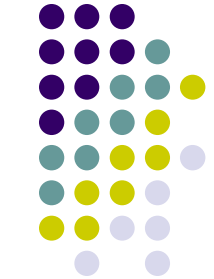
- At 4 hour time point, mean cell densities of Fn coated and TC treated plates are not significantly different
  - α = 0.05 and P value = 0.13

	Fn Coated	*TC Treated*
Mean	4333.333	3466.667
Variance	303333.3	253333.3
t Stat	2.490348	
P(T<=t) one-tail	0.065215	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.13043	
t Critical two-tail	4.302653	

Conclusions on HDF Viability

- Concentration of cells in active metabolism is directly correlated to absorbance at 570 nm
  - Relationship is Abs. = $2*10^{-5}*$(Cell Concentration)-0.0186
- 250 μL of ethanol kills 100% of cells in one1 mL of a 50,000 cell/mL cell suspension
  - Ethanol changes cell membrane permeability to specific dyes
  - 10 μL of ethanol also causes significant cell death
- Cell viability established by differences in the action of specific chemicals between living and dead cells
  - Differences highlighted by absorbance or fluorescence

Conclusions on HDF Attachment



- HDFs exhibit more spreading and extension of pseudopodia on Fn coated surfaces
- Quantitative data implies:
  - Greater total HDF attachment to Fn coated plates at each time point
  - Increased attachment rates to Fn coated plates over 4 hour time period
- Overall results suggest Fn coated surfaces facilitate cell adhesion