

# Factors Contributing to Human Dermal Fibroblast (HDF) Cell attachment and viability

# Objectives

- Assess toxic effects of media on viability of living cells
  - Live/Dead Fluorescence Assay
- Determine affects of surface on cell attachment
  - Fibronectin Attachment
  - Quantitative Cell Attachment

# Live/Dead Assay Methods

- HDF cells are seeded onto TC Treated well plates
- Following 2 day 37°C incubation, cells are rinsed with PBS, new environmental condition is applied (PBS, ethanol, or PBS +3 drops ethanol)
- Cells are dyed with Ethidium Homodimer/Calcein AM dye
- Cells are then observed under light and fluorescent microscopes

# Fibronectin Attachment Methods

- 50,000 HDF cells/well are seeded onto Fibronectin (Fn) coated plate and non coated plate
- Following a 2 hour incubation, cells are observed using a light microscope to determine differences in cell attachment before and after rinsing with PBS

# Quantitative Cell Attachment Methods

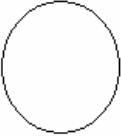

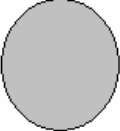
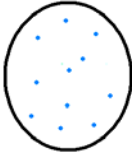
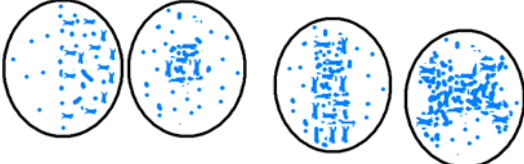

- 10,000 HDF cells/well) are seeded onto
  - Fn coated plate
  - TC-treated plate
  - Non-coated
- Number of attached cells counted 4 times within 4 hours using a light microscope

# Ethanol is Toxic to Cells

- Dye stains dead cells red, but live cells remain green under fluorescent microscope

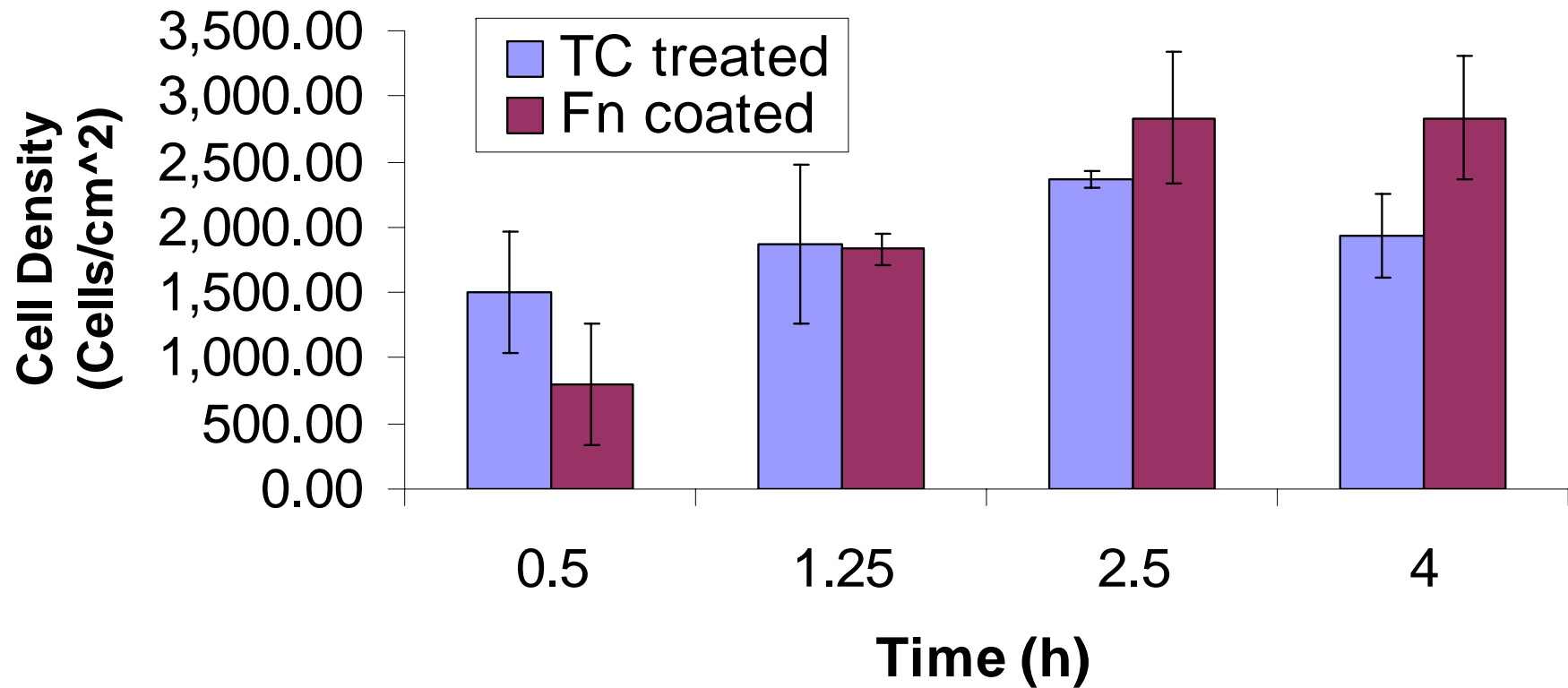
| <b>Condition</b>                      | <b>Observation</b>  |
|---------------------------------------|---|
| 250 $\mu$ L PBS, dye                  | Nearly all cells are green and elongated, with a few red, round cells |
| 250 $\mu$ L ethanol, dye              | All cells are red and round   |
| 250 $\mu$ L PBS, 3 drops ethanol, dye | Most cells are green, but scattered areas of red cells are present    |

# Surface Affects Cell Attachment

| Fibronectin Coating        | Control   | Patterns test conditions   | Completely Fn covered   |
|----------------------------|---|--|---|
| Observation before rinsing |    |    |    |
| Observation after rinsing  |  |  |  |

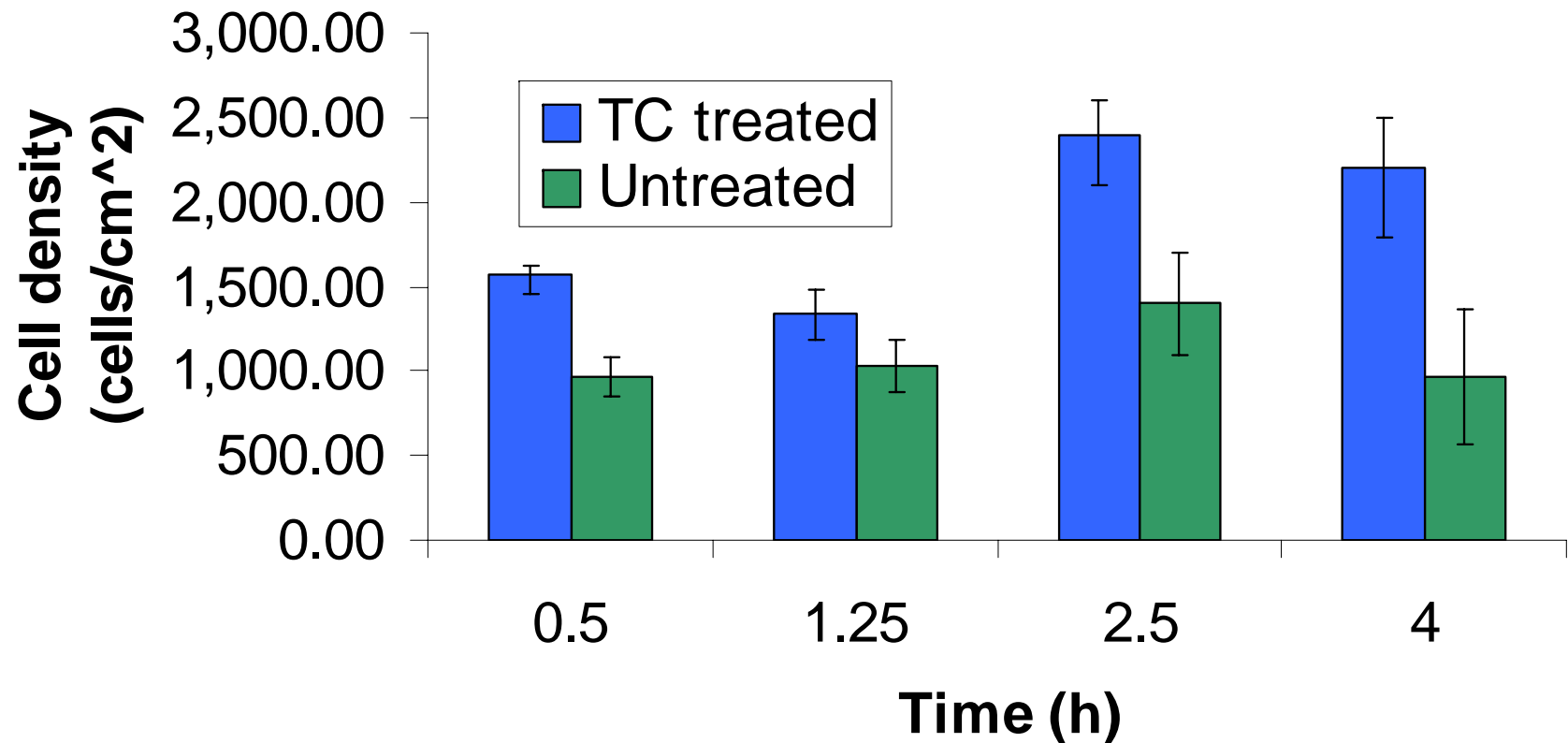
- Cells are attaching more on Fibronectin coated areas.
- Difficult to observe before rinsing unattached cells

# Cell Attachment is greater for Fn coated





# Cell Attachment is greater for TC treated



Data from \*\*\*\*\*

# Cell attachment is surface dependant

- Difference in Cell attachment at 4 hours
  - Fn Treated is greater than TC Treated ( $p=.05$ , t-test)
  - TC Treated is greater Untreated ( $p=.01$ , t-test)
- Elongation/Cell Spreading
  - Fn Coating > TC Treated for all times
  - TC Treated > Untreated for all times

# Data from Fn Attachment assay and Quantitative Cell attachment agree

- Concentration
  - Qualitative observation demonstrates that the highest density of attached cells occurs on Fn coated areas.
  - The increase in cell density on fibronectin within 4 hours supports this conclusion (Quantitative)
- Morphology
  - It can be seen in both quantitative and qualitative experiments that Fn coated areas have more elongated cells

# Environmental factors impact HDF cell attachment and viability

- There is increased cell density on Fn Coated surfaces
  - Fn coated density > TC treated and TC treated > Nontreated surfaces
- Ethanol contact leads to death of HDF cells