Viability and Attachment of Human Dermal Fibroblast (HDF) cells *in vivo*

YYY
BIOE 342
Rice University

Statement of purpose

- To qualitatively observe cell attachment and morphology of HDF cells to fibronectin-coated (Fn) surface
  - Fibronectin Attachment Assay
- To quantitatively observe the rate and degree of HDF cell attachment to TC-treated, untreated, and Fn-coated surfaces
  - Quantitative Cell Attachment Assay
- To assess the impact of ethanol and PBS on the viability of HDF cells using fluorescent staining procedures
  - Live/Dead Fluorescence Assay

Fibronectin Attachment Assay Methods

- HDF cells seeded at uniform concentration onto non-TC treated wells under four test conditions
  - Condition A: (control) No fibronectin
  - Condition B: Half fibronectin
  - Condition C: Pattern-painted fibronectin
  - Condition D: Fibronectin
- After 2-hour incubation, observed cell attachment and morphology using a light and fluorescent microscope before and after PBS wash

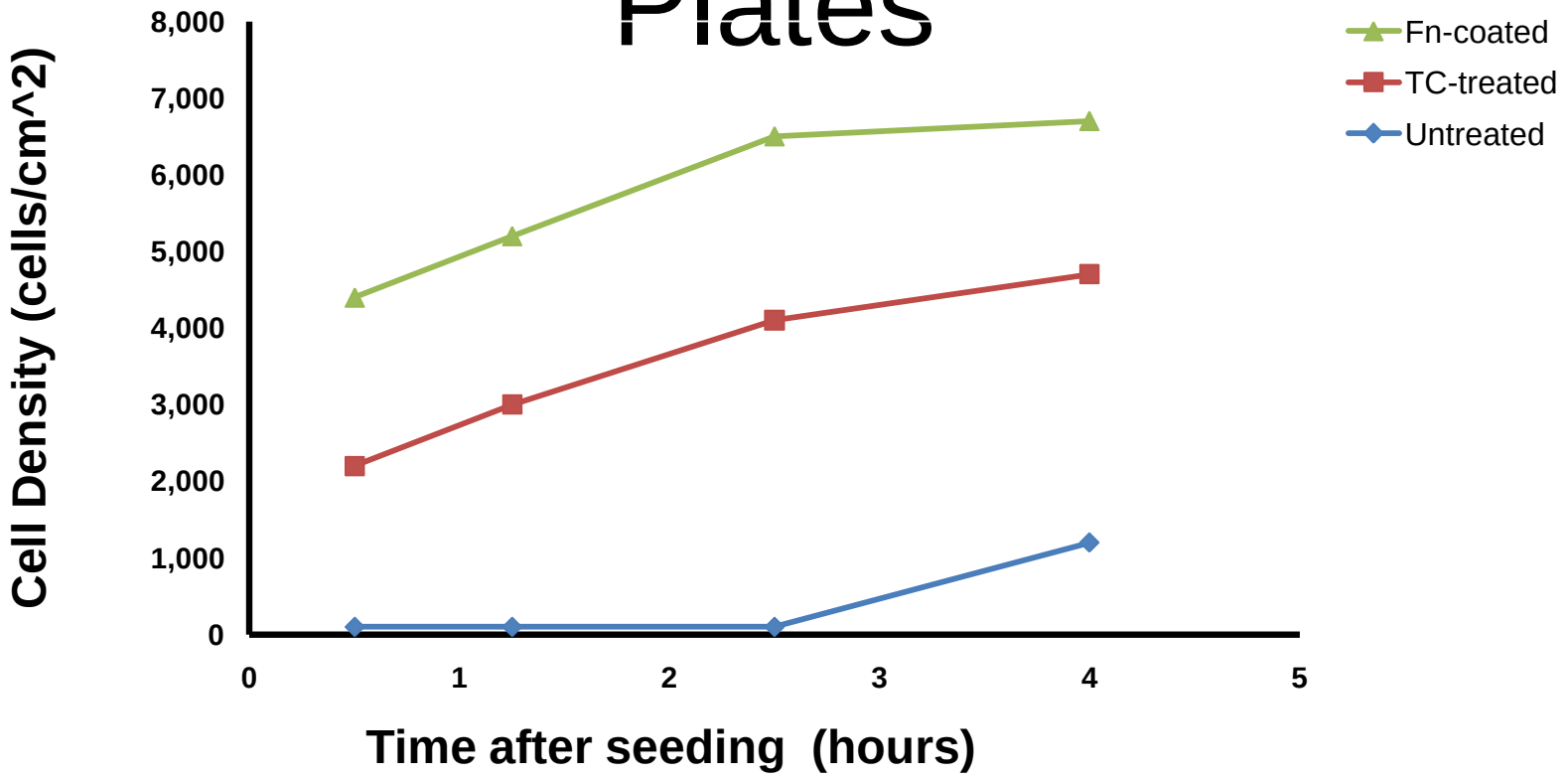
Fibronectin promotes cell attachment and spread morphology

	Before Wash		After Wash	
Test Condition	**Morphology**	**Attachment**	**Morphology**	**Attachment**
A: No Fn	None spread out All rounded cells	None	No cells	No cells
B: Half Fn	(Fn side) All spread (non-Fn) All rounded	On Fn-half	Spread cells on Fn-side No cells on non-Fn-side	On Fn-half
C: Pattern-painted Fn	(along Fn-pattern) Most spread out Some rounded	Along Fn-pattern	Spread cells along pattern	Along Fn-pattern
D: Fn	All spread out None rounded	All over	Spread cells all over	All over

Quantitative Cell Attachment Assay Methods

- HDF cells seeded at uniform concentration onto untreated, TC-treated, and Fn-coated polystyrene well plates
- Wells were incubated for 0.5, 1.25, 2.5, and 4 hrs
- After three PBS washes, determined cell density by counting attached cells in 10x10 grid of light microscope

Cell Density of HDF Cell Attachment Greater for Treated Plates



- Fn-treated plate had higher rate and degree of cell attachment; untreated plate had lowest

Live/Dead Fluorescence Assay Methods

- After seeding HDF cells at uniform concentrations onto TC-treated plate and 2-day incubation, created three test conditions
  - Condition A: PBS (control-no ethanol)+ dye
  - Condition B: Ethanol + dye
  - Condition C: PBS + ethanol (2 drops) + dye
- Observed morphology color of cells and cell nuclei using light and fluorescent microscope

Ethanol (Toxic Material) Kills HDF Cells

Treatment	Toxicity	Morphology	Color	Viability
A: PBS + dye	No	Elongated and stretched	Cells stained green	Live
B: Ethanol + dye	Yes	Small, rounded cells	Cell nuclei stained red	Dead
C: PBS + ethanol (2 drops) + dye	Yes	Both rounded and elongated cells	Both green-stained cells and red-stained nuclei	Live and Dead

- Light microscope used for morphology, fluorescent used for color
- Live cells are seen as green through the fluorescent microscope; dead cells' nuclei are seen as red
- Ethanol is toxic to cells and kills them by lysing their nuclei

Cell Attachment Synthesis

- Fibronectin Attachment Assay
  - Fn promotes cell attachment and spread morphology of HDF cells
- Quantitative Cell Attachment Assay
  - HDF cells have a higher degree and rate of attachment for Fn-coated and lower for untreated surfaces
- Treated cells promote HDF cell adhesion and spread morphology. Fn has a higher degree and rate of attachment than TC-treated surfaces.

Conclusions

- HDF cells attach to treated cells over untreated cells
  - Fibronection is an acceptable protein for promoting cell attachment and spread morphology onto untreated surfaces
- The spread morphology is indicative of a live cell
- Toxic materials such as ethanol kills cells, changing their morphology from elongated to rounded cells