Adhesive Properties and Viability of Fibroblasts Exhibit Substrate Dependency

YYY

11 February 2009

Objectives

- Determine ability of Human Dermal Fibroblasts (HDF) to adhere to varying substrates.

- Assess cell viability on substrate exhibiting greatest adhesion.

Cell Attachment Assay Methods

- HDF cells were seeded in DMEM + 10% serum + 1% antibiotic on three unique substrates at 10,000 cells/mL:
  - tissue culture (TC)-treated, untreated, and Fibronectin (Fn)-coated plates.
  - target cell concentration = 5000 cells/mL
- Average cell density was calculated via light microscopy.
  - count area of 0.01cm^2

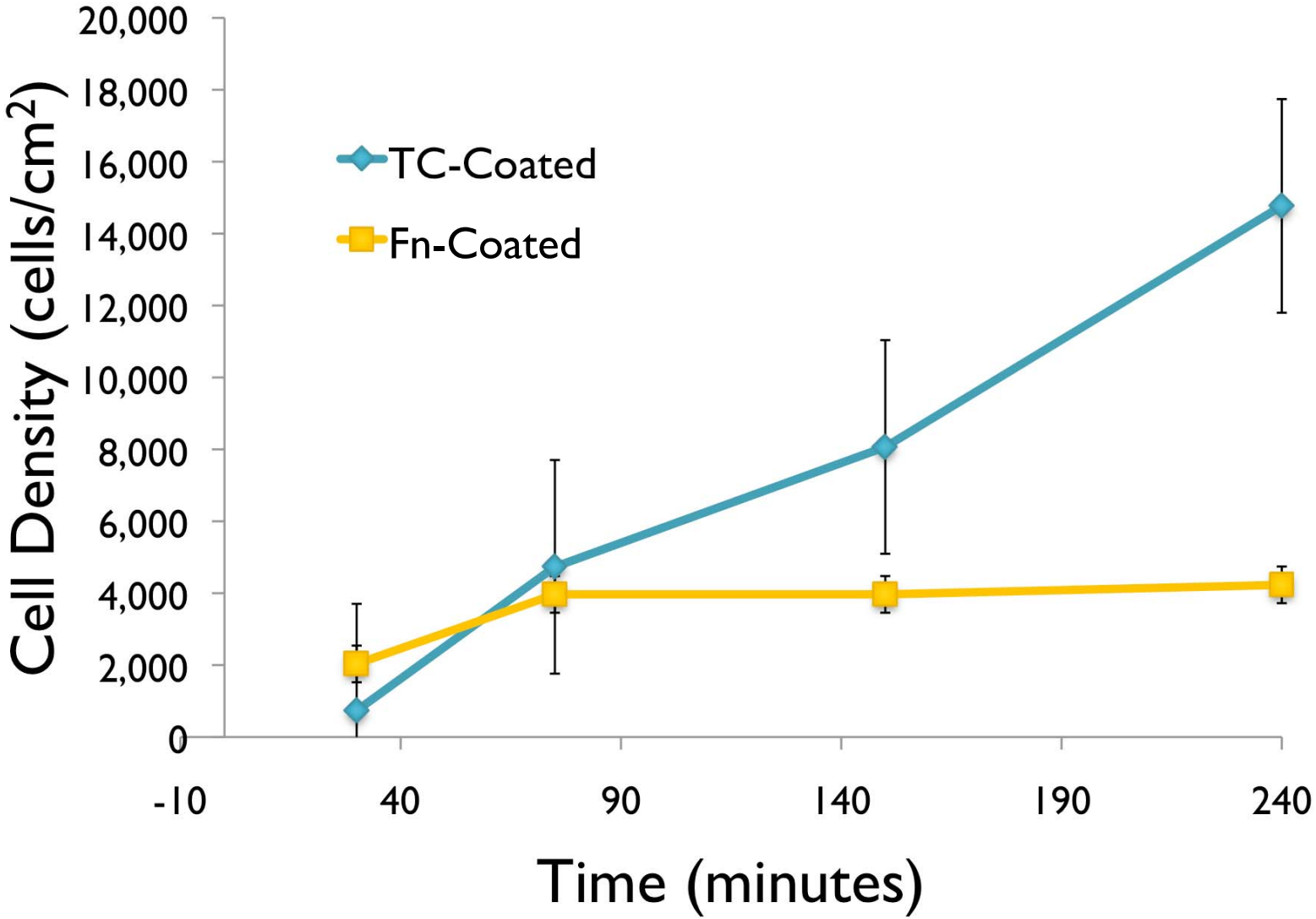
Fibronectin Attachment Assay Methods

- HDF cells were seeded in DMEM on non-TC-treated wells partially painted with fibronectin (Fn).
  - target cell concentration = 50,000 cells/mL.
  - control wells were left unpainted.
  - Fn painted in "X" pattern on experimental wells.
  - incubation time of two hours
- Cell adhesion was assessed via light microscopy.

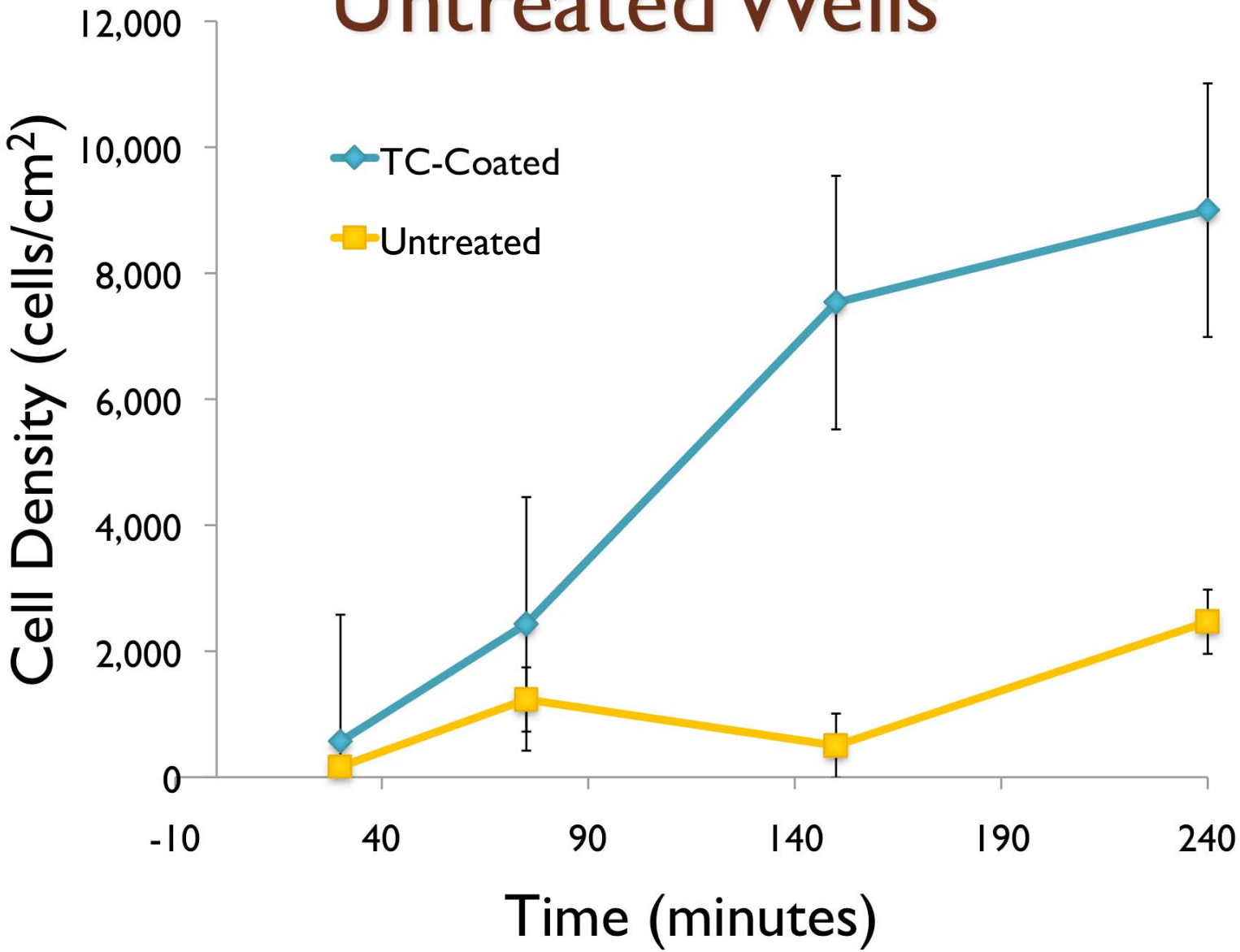
MTT Viability Assay Methods

- HDF cells were seeded in DMEM + 10% serum on TC-treated plates.
  - seeded concentration of 0 to 50,000 cells/mL.
  - incubation time of two days.
- MTT dye will stain cells that are metabolizing
  - will provide correlation between dye absorbance and cell viability.

HDF Adhesion Is Greater On TC-Treated Wells Than Fn-Coated



HDF Adhesion Is Minimal On Untreated Wells

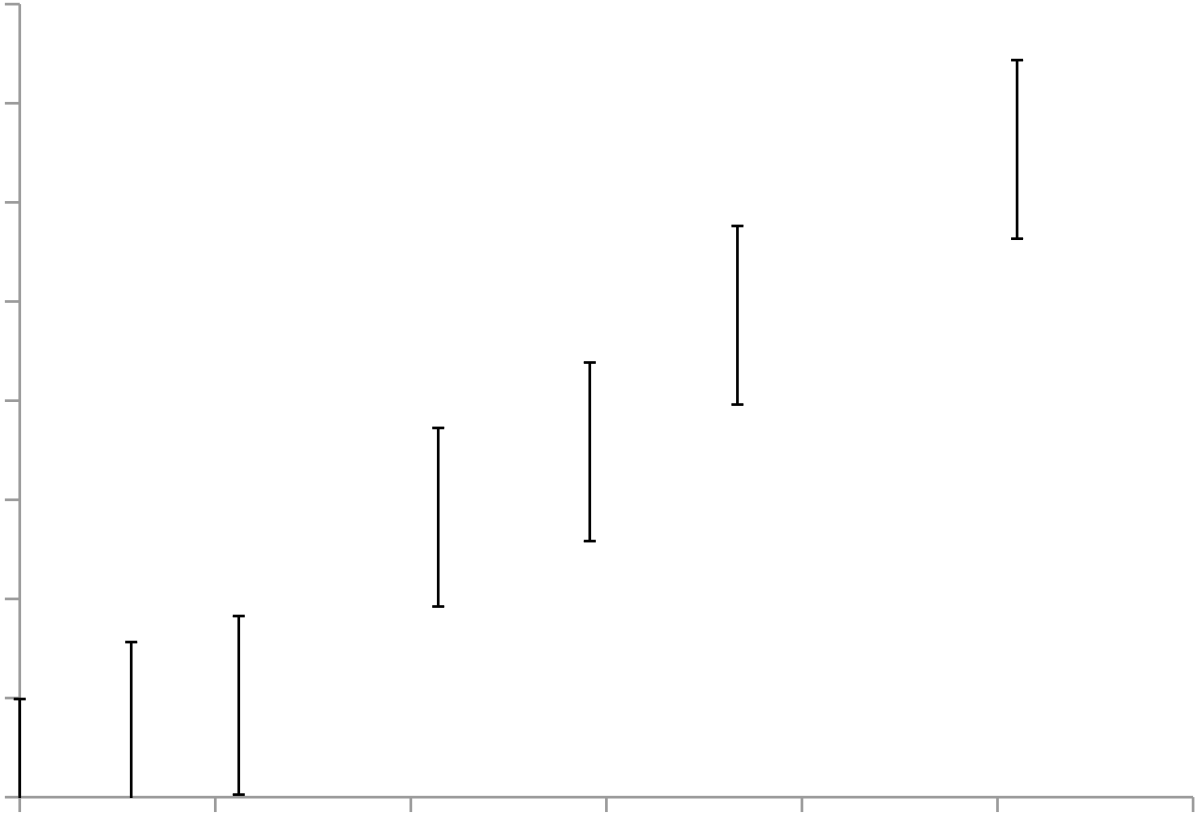


HDFs Change Morphology Upon Adhesion to Fibronectin

- Fn-coated wells contained more flat, spread, two-dimensional fibroblasts.
  - cells were attached properly to Fn substrate and no longer floating in solution.
- Fibroblasts adhered to specific location of well with painted Fn.
  - cells located on painted "X" were more attached and spread
  - cells on unpainted regions were detached / round.

Sample well painted with Fibronectin "X" pattern.

Higher Absorbance Correlates With Increased Viability



HDF Attachment Is Dependent Upon Substrate

- Fibroblast adhesion is greatly increased by specific and non-specific interaction.
  - TC-treated wells allow charge-charge non-specific interaction to initiate cell adhesion.
  - Fibronectin-coated wells offer cells a substrate simulating an extracellular matrix environment with Fn-Integrin binding.
  - Untreated wells of plain polystyrene proved least effective at initiating HDF adhesion.

Integrating HDF Viability, Proliferation, And Adhesion

- Fibroblast viability correlates with MTT dye absorbance.
  - This method proved effective with TC-treated wells.
  - Future viability experiments can include cells seeded on untreated and Fn-coated wells.
    - This would allow direct correlation between substrate and cell viability.

Cell Proliferation Adds Additional Facet To Viability

- Previous work displayed exponential cell growth over seven days.
  - Proliferation experiments with Fn-coated and untreated plates would provide new data correlated with MTT assays.

Experimental data was obtained in BIOS 320-002 laboratory. Cell attachment data corresponding to TC-treated versus uncoated plates was obtained from partner XXX.