Effects of Culture Conditions on Attachment and Viability of Human Dermal Fibroblasts

YYY

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Objective

- Qualify and quantify attachment of HDF cells on TC-treated, fibronectin-coated (Fn), and untreated polystyrene
- Explore cell viability
  - Examine effects of ethanol on viable cells
  - Graphically observe the relationship between cell concentration and cell viability

Qualifying Cell Attachment

- Untreated polystyrene wells were half, fully, or selectively coated with fibronectin or left uncoated; all were incubated for 30 minutes.
- Wells were rinsed in PBS with Bovine Serum Albumin (BSA).
- 50,000 cells were placed in straight DMEM on each well and incubated for 2 hours.
- Regions of cell attachment were determined in phase contrast.

Quantifying Cell Attachment

- 10,000 cells per test well were placed in DMEM with 10% FBS on untreated, Fn-coated, and TC-treated polystyrene.
- Wells were rinsed to remove free cells at 30, 75, 150, and 240 minutes of incubation, and remaining cells were counted in phase contrast.

Plate Treatments Improve Fast Attachment

- More HDFs attach within the first 150 minutes following seeding on treated than untreated polystyrene.
- In a well with regions of raw and Fn-coated polystyrene, cells attach only to the coated areas within 2 hours of seeding.

Ethanol on Viable Cells

- 75,000 cells were plated in each well of a TC-treated polystyrene plate and incubated for 2 days in 10% FBS DMEM.
- Wells were covered in 70% ethanol completely, partially, or not at all.
- Live and dead cells were differentiated using fluorescence microscopy with Calcein AM and Ethidium Homodimer dyes.

Viability and Concentration

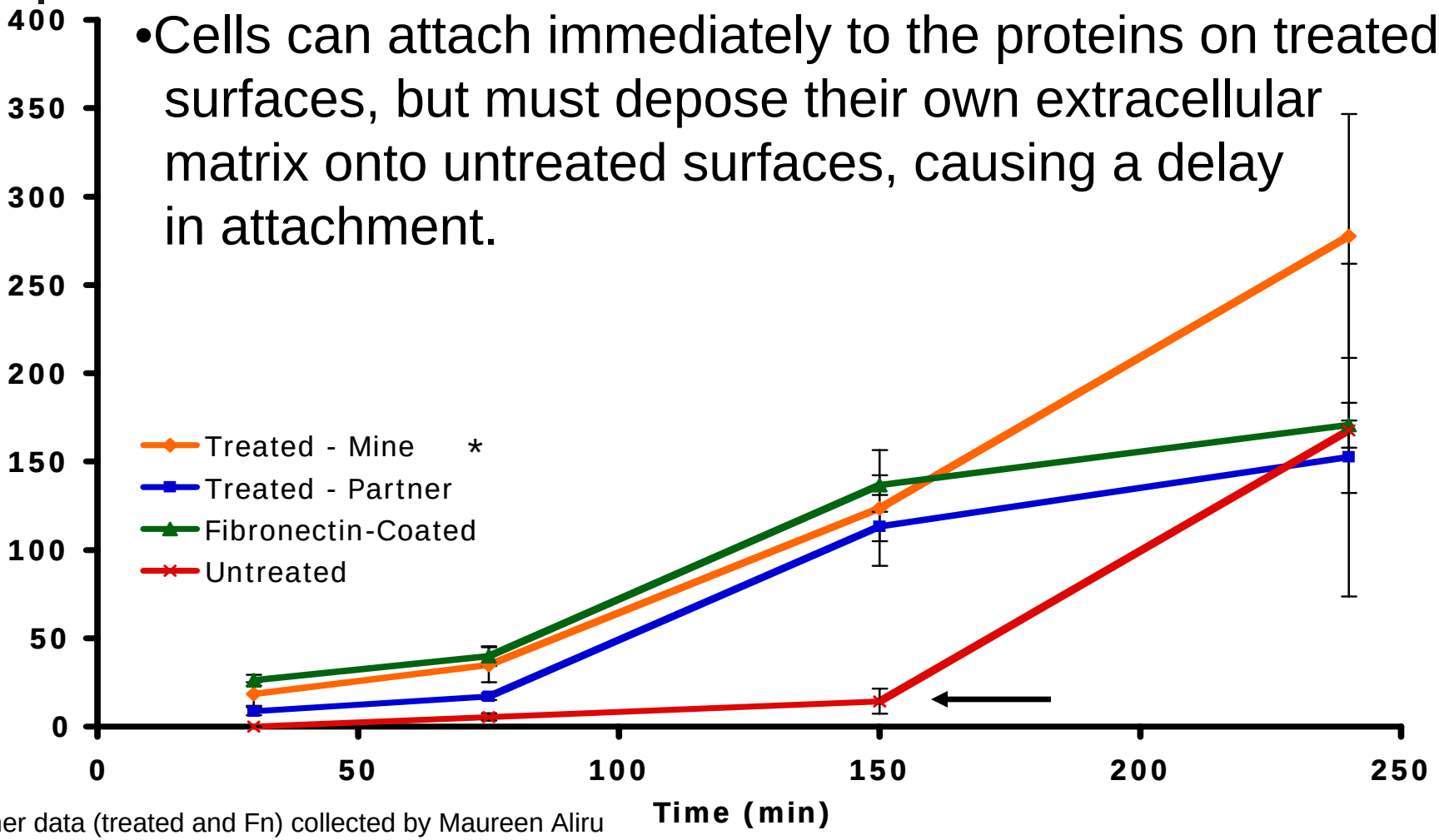
- Cells were seeded in set dilutions, from 25,000 to 0 cells per well, into two identical plates of TC-treated polystyrene wells in 10% FBS DMEM, and were incubated for 2 days.
- One plate's wells were trypsinized, and cell count was determined via Coulter Counter.
- The other plate's wells were incubated with MTT dye for 2 hr, stopped, and had their absorbances read with a spectrophotometer.
- Raw count data was plotted against absorbance data to find a relationship.

Fibronectin Allows Attachment

- After 2 hours of incubation, cells were evenly distributed in wells but had only attached to regions that had been coated in Fn before incubation.
- Cells had elongated morphologies with psuedopodia stretching along and defining the edges of coated regions.

Treated Surfaces Promote Fast Cell Attachment

- Cells can attach immediately to the proteins on treated surfaces, but must depose their own extracellular matrix onto untreated surfaces, causing a delay in attachment.



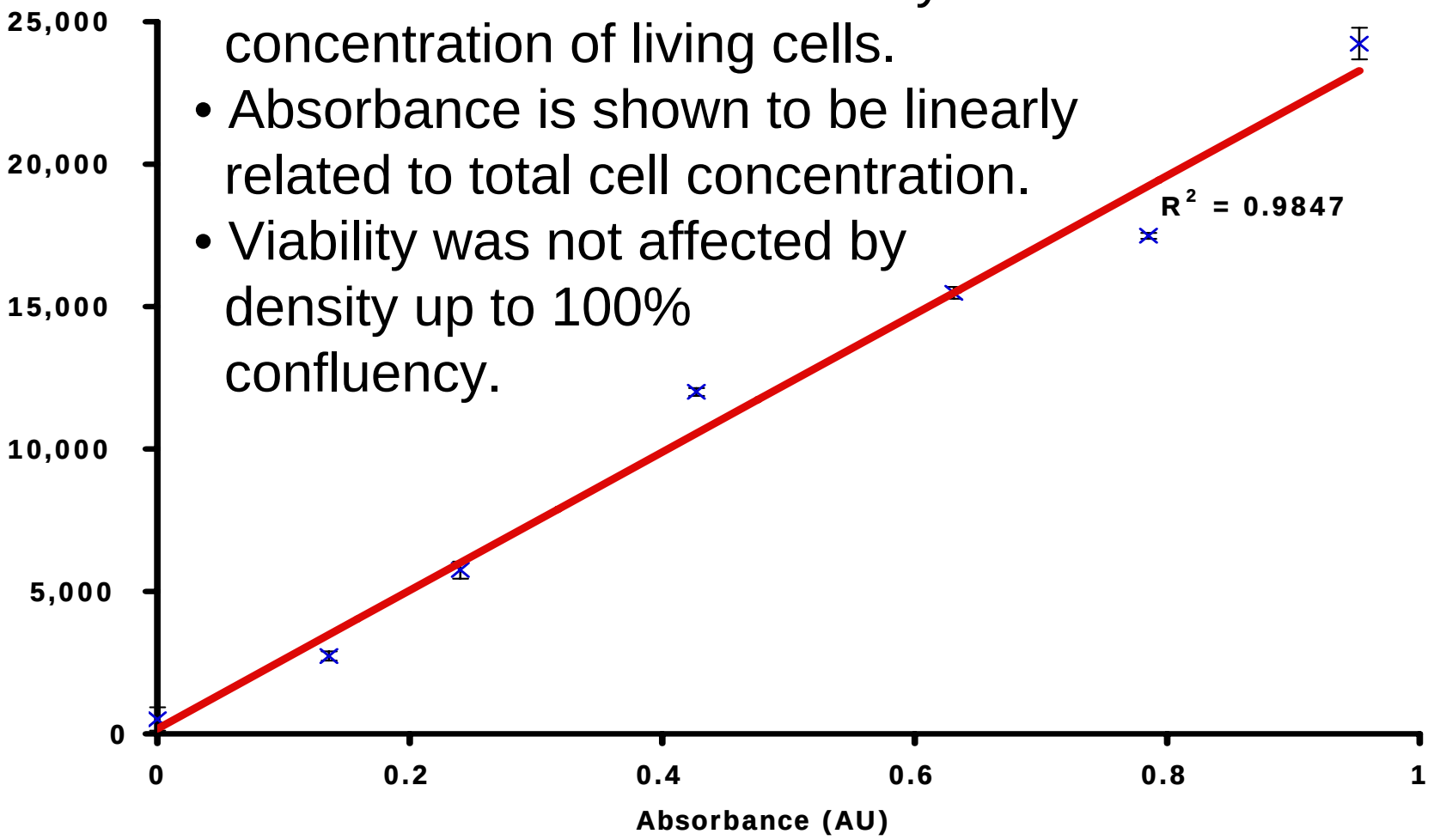
* Partner data (treated and Fn) collected by Maureen Aliru

Ethanol is Toxic to Cells

- Healthy cells that were not exposed to 70% Ethanol (EtOH) were nearly 100% viable at high confluency.
- Viability in wells given two drops of 70% EtOH was about 60% lower than healthy cells at high confluency.
- Healthy cells covered with 70% EtOH lost all viability.

Viability is Independent of Attached Cell Density

- Absorbance in an MTT assay is a measure of the concentration of living cells.
- Absorbance is shown to be linearly related to total cell concentration.
- Viability was not affected by density up to 100% confluency.



Quick Attachment Dependent on Plate Conditions

- Treatments accelerate attachment on polystyrene.
  - TC- and Fn-treated plates more quickly attach HDF within 150 minutes of seeding.
  - HDFs attach Fn-coated but not blank polystyrene within 2 hours of seeding.

Viability Independent of Density; Subject to Toxins

- Viability is independent of attached cell density.
- 70% ethanol is toxic to HDF cells.