HDF Survival and Function

Tissue Culture Lab
BIOE 342

Human Dermal Fibroblast Testing

- Fibronectin Attachment Assay
  - To qualitatively assess the attachment of HDF cells to un-treated and fibronectin (Fn) coated surfaces
- Quantitative Cell Attachment Assay
  - To quantitatively assess attachment of HDF cells to tissue culture (TC) treated, un-treated, and Fn-coated surfaces
- Cell Proliferation Assay
  - To quantitatively assess the effects of serum on the growth and replication of HDF cells

Fibronectin Attachment Assay

- Non-TC treated polystyrene plates prepared (2 wells/condition):
  - Control: 500µL of PBS
  - Half Fn/ Half control: 3 coats of Fn solution painted on half of well
  - Design: Letter 'X' painted with Fn solution
  - Fn coated bottom: 300 µL Fn aliquots into wells, wetting entire bottom.
- Plates incubated for 30 minutes.
- Wells rinsed with 1mL PBS containing 10mg/mL BSA.
- 1mL of 50,000 cells/mL cell solution in DMEM (no serum or antibiotics) seeded into each test well.
- Plates incubated for 2 hrs.
- Cell adhesion, morphology, and attachment pattern checked using a light microscope.
- Aspirated supernatant and washed with PBS.
- Cell adhesion, morphology, and attachment pattern observed using a light microscope.

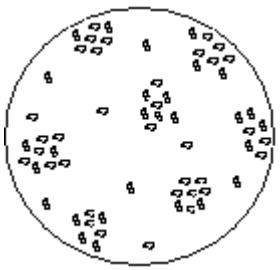
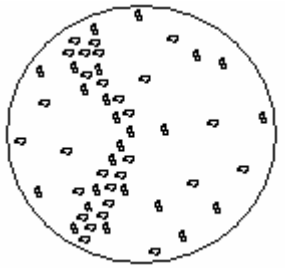
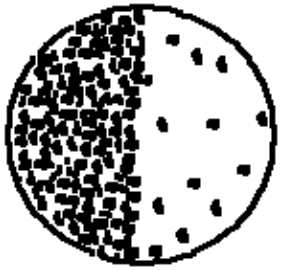
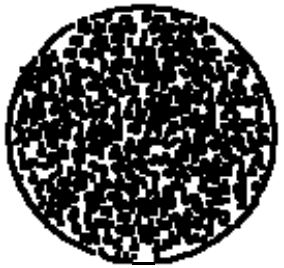
Quantitative Cell Attachment Assay

- Seeded 1 mL of 10,000 cells/mL cell suspension in DMEM with 10% serum and 1% antibiotic into 8 wells on each well plate with TC-treated, Fn-coated, or untreated surface.
- Plates incubated and removed after 30 minutes, 1hr 15 minutes, 2hrs 30 minutes, and 4 hrs for observation:
  - At observation time, washed two wells in each condition with PBS.
  - 1mL PBS added to each well for counting procedure.
  - Observed wells under a light microscope with a 10X objective.
  - Counted cell number in 10x10 grid and calculated cell density.
  - Observed shape, morphology, and distribution of cells.

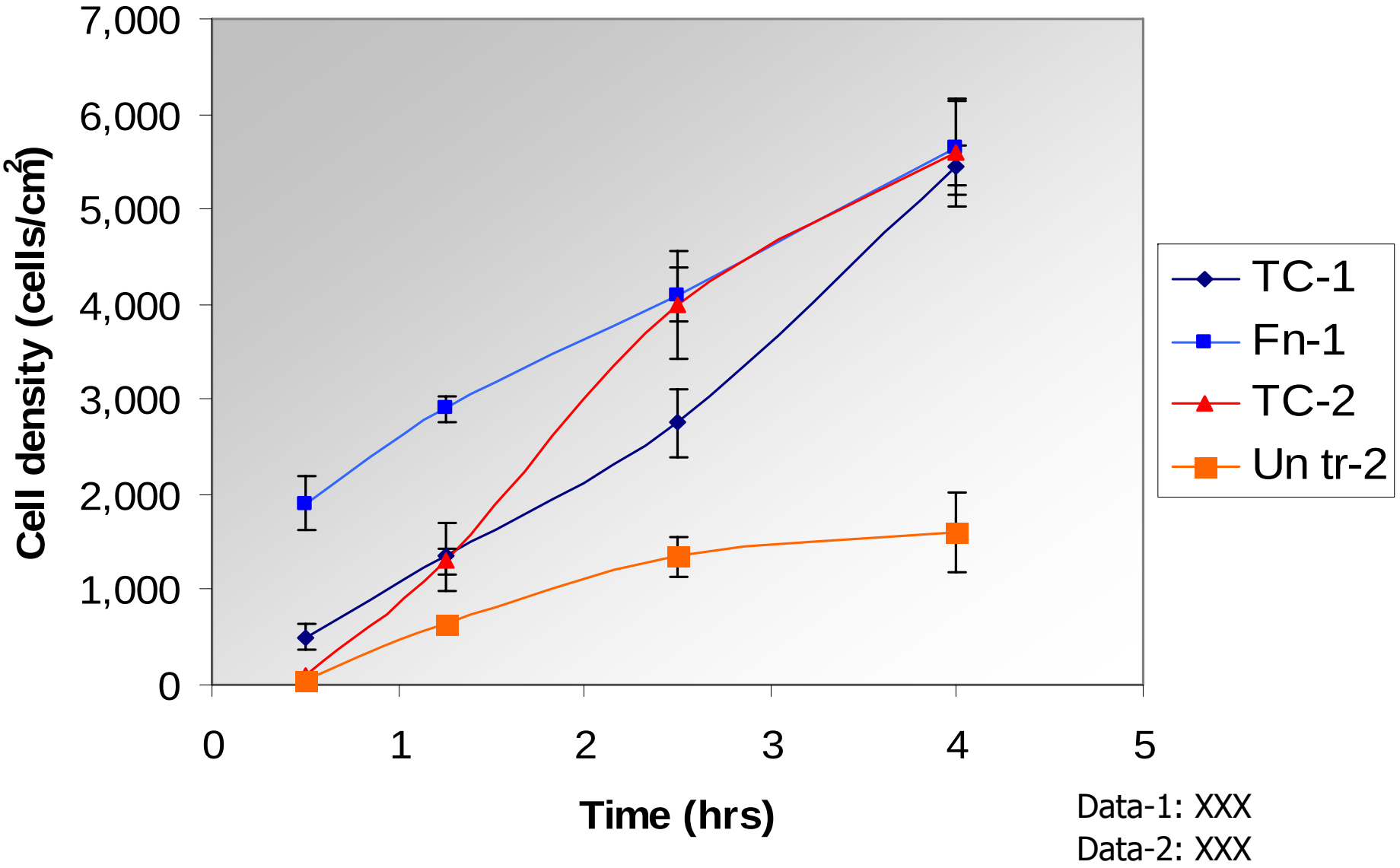
Cell Proliferation Assay

- Seeded 1mL of 7,000 cells/mL cell suspension in DMEM containing 1% serum into each test well.
- Incubated plates for 4 hours.
- Aspirated liquid media covering cells in wells.
- Added 1mL DMEM containing 1%, 5%, or 10% serum to appropriate sample wells.
- On Days 0-4hrs, 2, 5, and 7, counted the appropriate wells:
  - Rinsed cells in wells to be counted with PBS.
  - Added 250 µL of trypsin to each well
  - Incubated plates for 10 minutes.
  - Added 750 µL of DMEM with 10% serum to each well.
  - Transferred liquid from each well into a Coulter Counter vial containing 9 mL Isoton.
  - Determined cell concentration using Coulter Counter.
- Remaining wells replenished with the appropriate media and returned to the incubator.

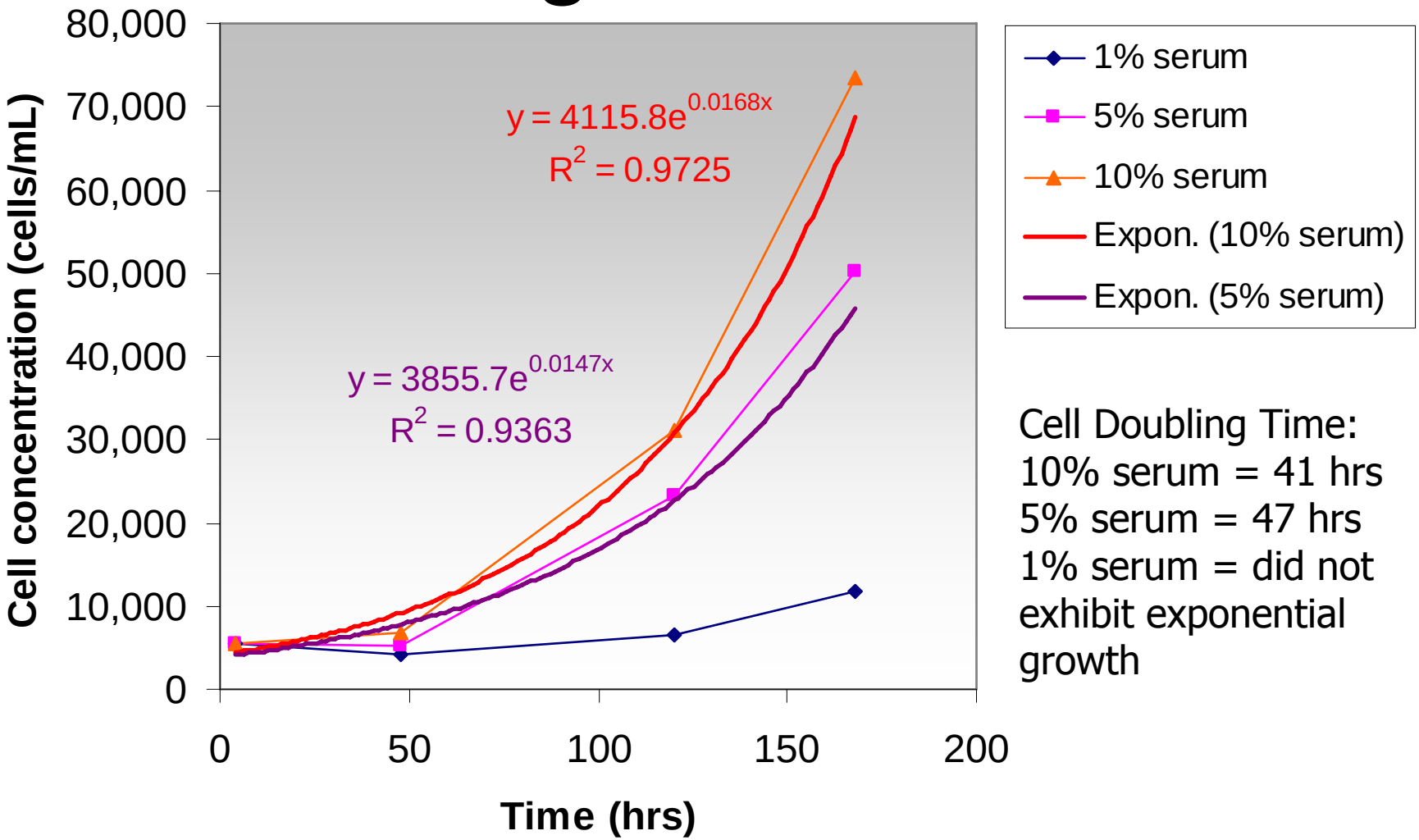
Fn Encourages Cell Attachment to Un-Treated Surfaces

After rinsing wells with PBS:	
Control: great reduction in cell number; few dense clumps remain 	**Design wells:** two legs of 'X' are visible; several cells still attached on the surrounding areas 
Half Fn/half control: cells on Fn side remain attached; very few cells left on control half 	**Fn coated bottom:** entire bottom still entirely coated with cells 

Surface Conditions Affect Cell Attachment



Cell Growth Rate Increases with Percentage Serum in Media



Cell Doubling Time:
10% serum = 41 hrs
5% serum = 47 hrs
1% serum = did not exhibit exponential growth

Cell Surface Attachment

- FAA: Fn facilitates the attachment of cells to untreated surfaces.
- Non-specific binding interactions to control surface discouraged by PBS containing bovine serum albumin wash.
- With weak cell to surface interactions, cell to cell interactions dominate and cells clump together rather than spread out across the surface.

Quantified Cell Attachment

- Fn-coated and TC-treated surfaces facilitate cell attachment.
- Surface conditions compared using an ANOVA test; not significantly different [F(1.466)<F crit(3.49)]
- TC-treated surface: partner rates not significantly different (t=-.143, p=.89)
- TC and Fn treated surfaces reached similar cell densities after 4 hours (~5,500 cells/cm^2).
- Untreated surface: never reached the cell density achieved by other conditions (~1600 cells/cm^2)

Cell Proliferation Rate

- Proliferation rate determined by amount of serum in media
- Cells depend on nutrition and growth factors in serum
  - 1% serum: cannot support cell growth
  - 5% serum: supports exponential cell growth
  - 10% serum: exhibits greatest exp cell growth
- Proliferation rates found to be significantly different using ANOVA test [f(1.794)>f crit(2.852)]

Qualitative vs. Quantitative Surface Attachment Analysis

- Both experiments compared treated and untreated well plates
- FAA: observations made visually; noted significant difference between Fn-coated and un-treated surfaces.
- QCAA: Quantitative measurements of cell densities support previous observations; Cell densities on treated surfaces more than twice the densities of untreated surfaces
- Demonstrates the importance of surface treatments when culturing cells

HDF Cells Dependent on Environment

- HDF cells in cell culture:
  - Attachment depends on surface treatments of container
  - Proliferation depends on the nutritional components of media.