

Surface conditions and media FBS  
concentration affect HDF attachment and  
proliferation *in vitro*

# Objectives

- To measure and compare fibroblast attachment over time on TC-treated, Fibronectin (Fn) coated and untreated polystyrene surfaces
  - Fibronectin is known to aid attachment *in vivo*
  - TC treatment is designed to enhance cell affinity
- To assess the relationship between media fetal bovine serum (FBS) concentration and HDF proliferation
  - serum contains many essential nutrients for cell survival

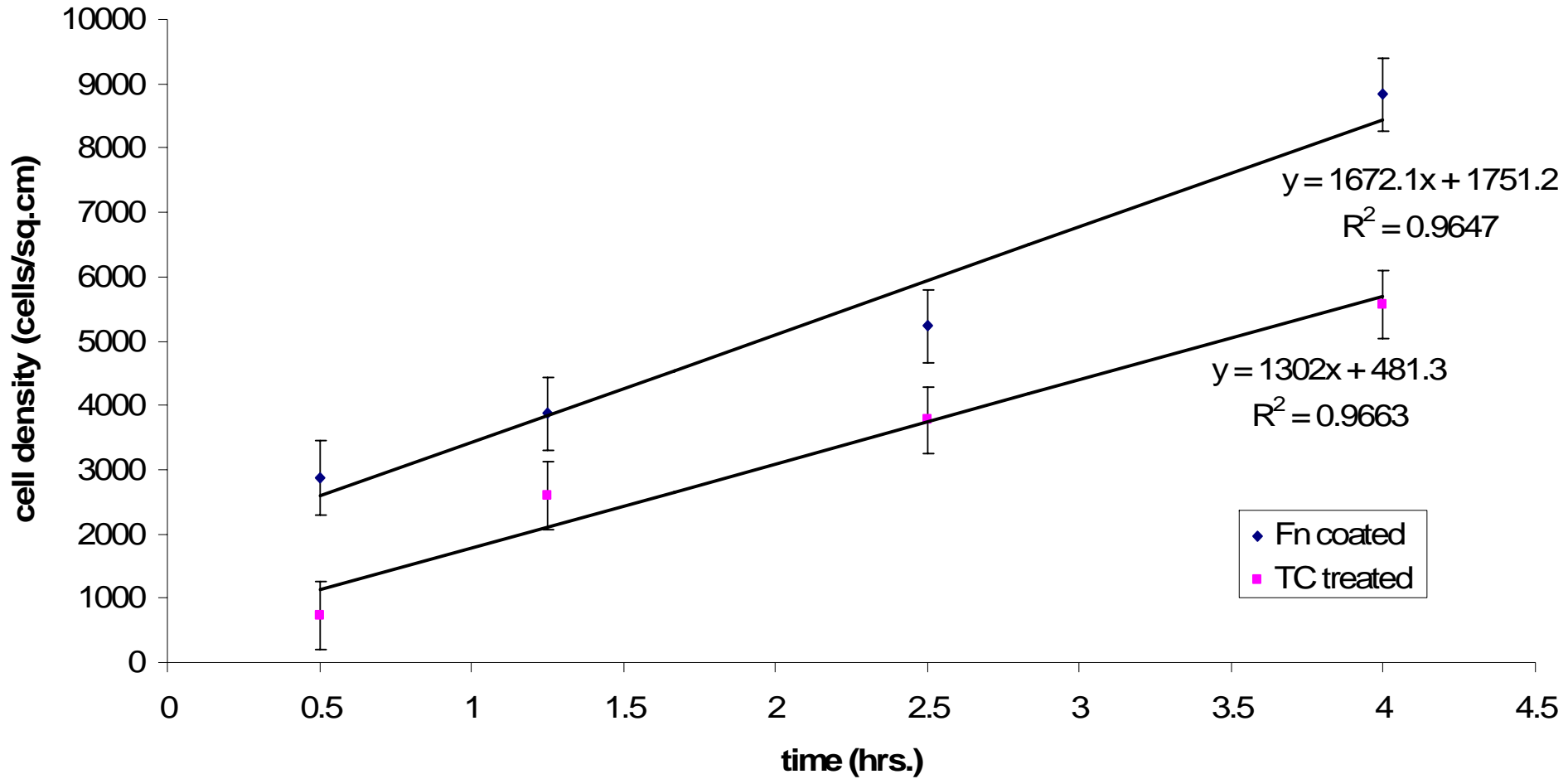
# Measuring Cell Attachment

- Cells were seeded in TC-treated, Fn-coated and untreated plates
- Plates incubated
- Wells rinsed with PBS to remove unattached cells
- Attached cell density taken from all plates at 30 min., 1 hr. 15 min., 2 hr. 30 min., and 4 hr. of incubation using light microscope

# Measuring How FBS affects Proliferation

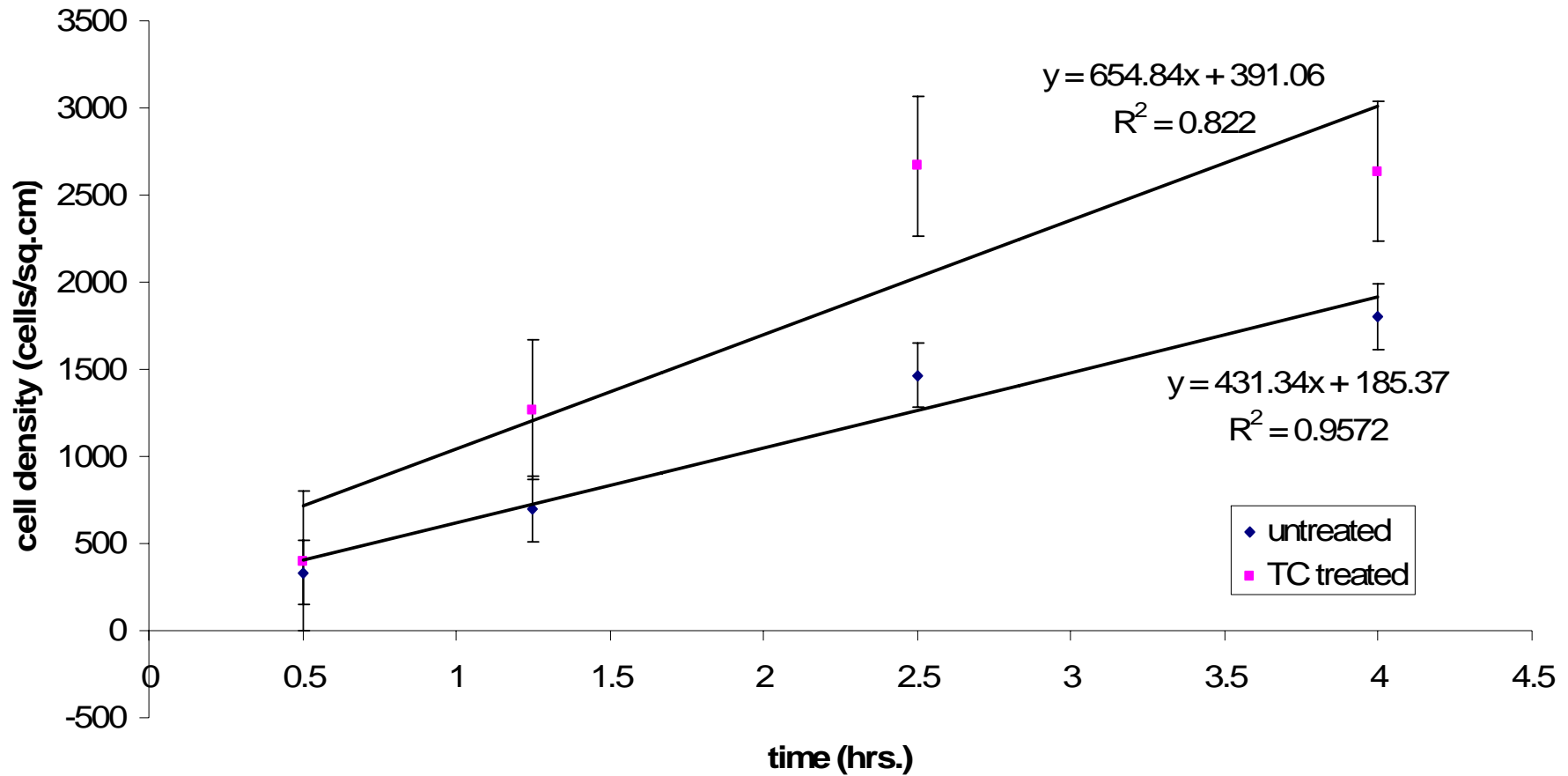
- Cells seeded on TC-plates in DMEM with 1%, 5% or 10% FBS
- PCNA assay- cells stain blue, proliferating nuclei stain red
  - cells in three FBS conditions incubated for 2 days
  - treated with Anti-PCNA-mouse IgG and Anti-mouse IgG-HRP
  - stained with AEC and hematoxylin
  - viewed under light microscope to measure confluency and percent of cells in S phase
- Proliferation assay
  - cells incubated for 2, 5 or 7 days for the three FBS conditions
  - cells counted at days 2, 5, and 7 with Coulter Counter

# Difference In Cell Density on TC and Fn Plates



P-value: 0.2673

# Difference In Cell Density on TC and untreated plates (data from partner)



P-value: 0.3514

Are there significant differences between cell attachment to experimental surfaces?

- Fn coating appears to facilitate cell attachment over TC treatment, and TC treatment appears facilitate attachment over untreated

- P-values indicate that differences are not significant

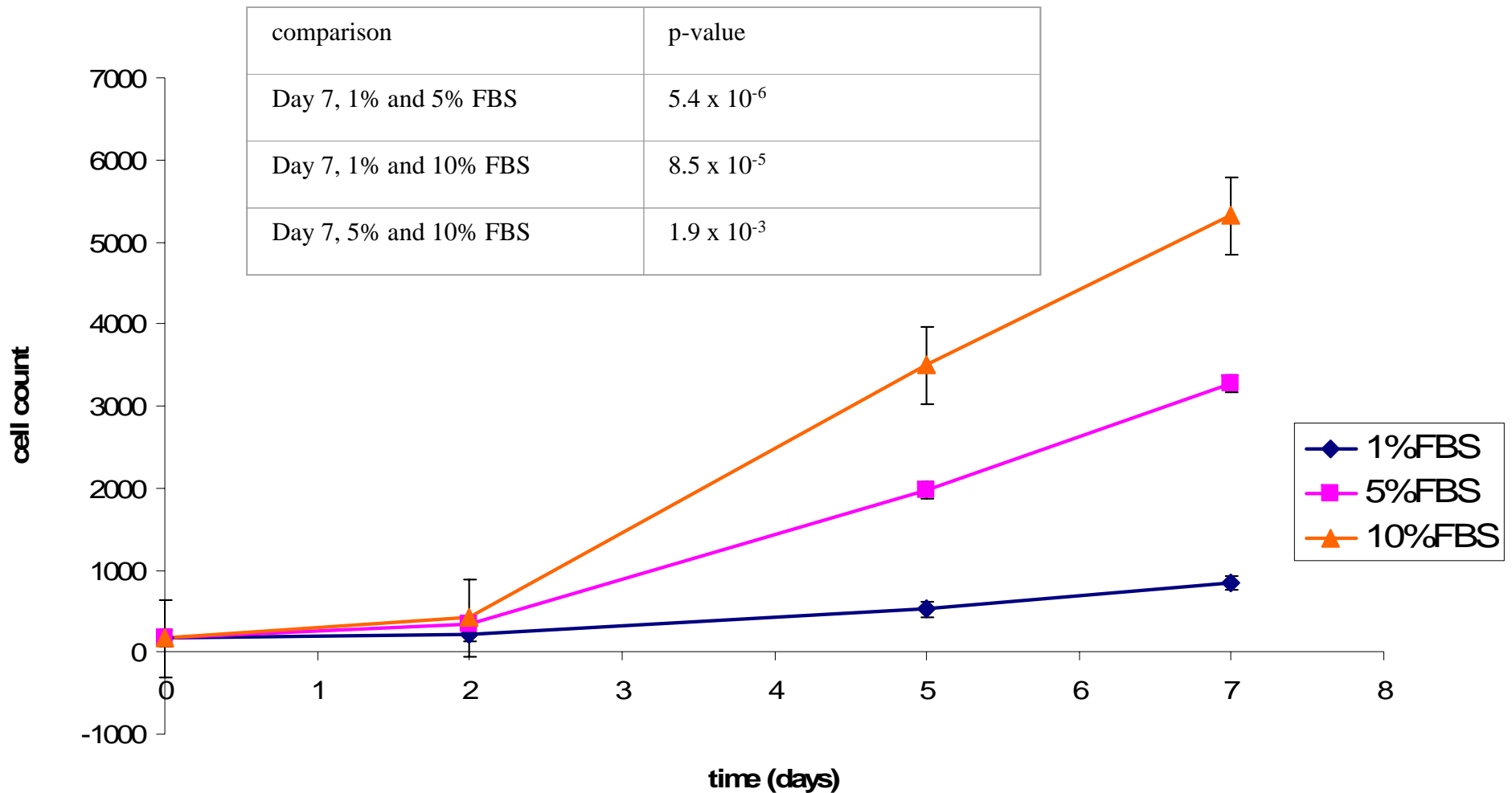
# Fraction in S Phase Depends on FBS Concentration

| <b>FBS Concentration</b> | <b>% S-Phase Cells</b> |
|--------------------------|------------------------|
| 1%                       | <10%                   |
| 5%                       | 70%                    |
| 10%                      | 100%                   |

- Fraction of cells in S phase is greatest for 10% FBS, comparable but lower for 5% FBS and much lower for 1% FBS

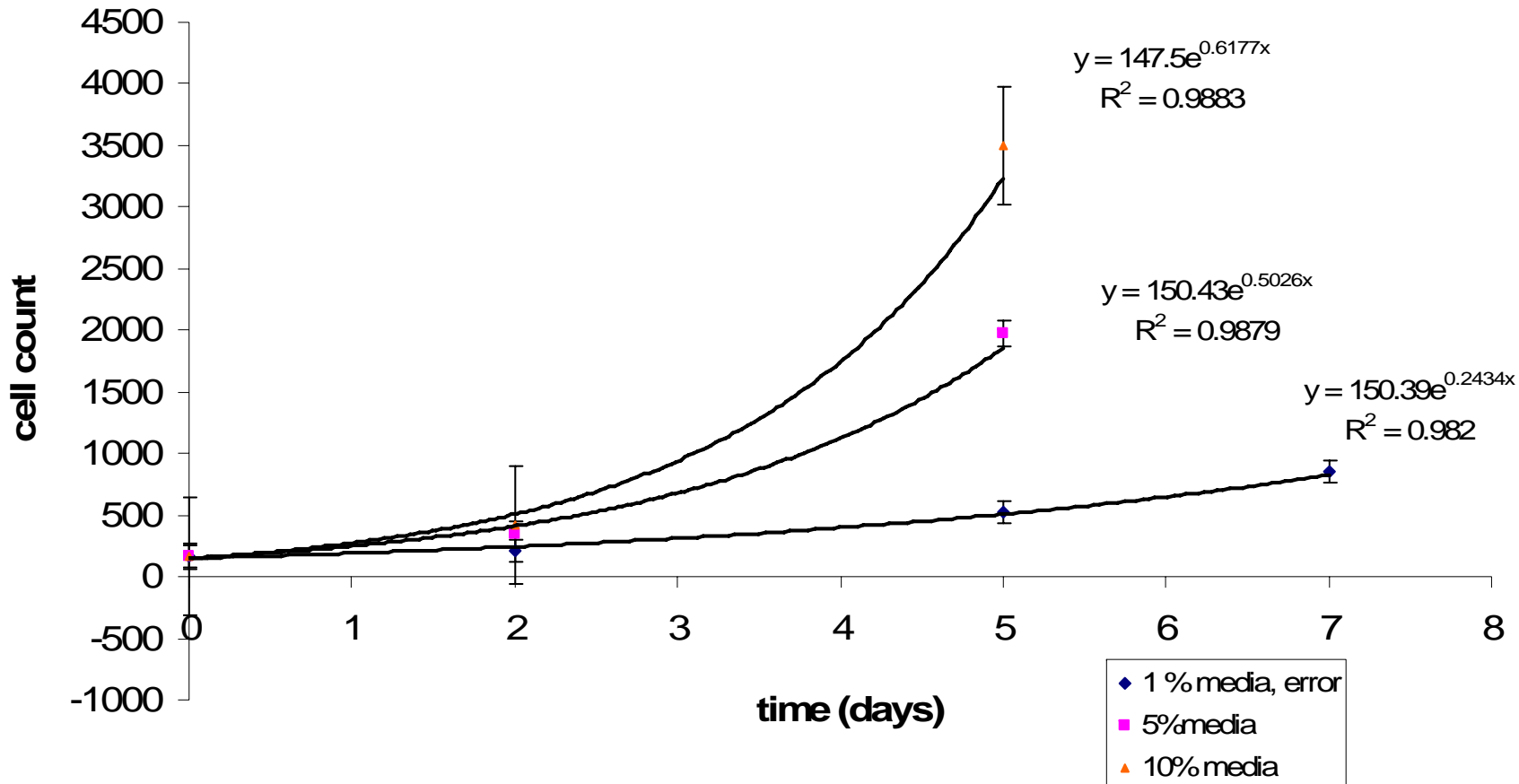


# Growth is Dependent on FBS Concentration



- There is a significant difference in cell count on Day 7 between any two experimental FBS conditions

# Doubling Time Depends on FBS Concentration



- Doubling times are: 1.13 days (10%), 1.38 days (5%), 2.85 days (1%)
- Doubling time for cells in 10% and 5% FBS are comparable, but growth rate for 1% FBS is much lower

# Growth Rate is Related to % of Cells in Synthesis

- At high FBS concentration, the percent of dividing cells is higher than at low FBS concentration
- At high FBS concentration, cells grow at a faster rate relative to at low FBS concentration
- Percent of dividing cells have a positive correlation with growth rate

# Conclusions

- TC treatment and Fn coating on a growth surface may enhance cell attachment, but there is no significant difference in cell attachment on either relative to non-treated plates (p values > 0.05)
- Sufficient FBS is necessary for the proliferation of cells. Growth rate at 5% FBS was relatively close to that at 10% while that for 1% was much slower.
- This was expected, since serum is known to have essential nutrients, but cells also have limited space and capacity to divide.
- Proliferation rate is proportional to fraction of S-phase cells
- This makes intuitive sense because proliferation rate is dependent on number of cell divisions in a given time