



Attachment and Proliferation of Human Dermal Fibroblasts (HDF)

YYY

2/10/09

BIOE 342



Objectives

- Compare HDF attachment on Tissue Culture (TC) treated surfaces with non-TC treated surfaces
- Determine effect of Fetal Bovine Serum (FBS) on HDF proliferation




Cell Attachment Assay Method

- TC and non-TC treated 24-well plates seeded with $1.0E4$ cells/well
- Cells incubated for 0.5, 1.25, 2.5, and 4 hrs
- Wells rinsed in saline before data collection
- Attached cell density measured using light microscopy



Cell Proliferation Assay Method

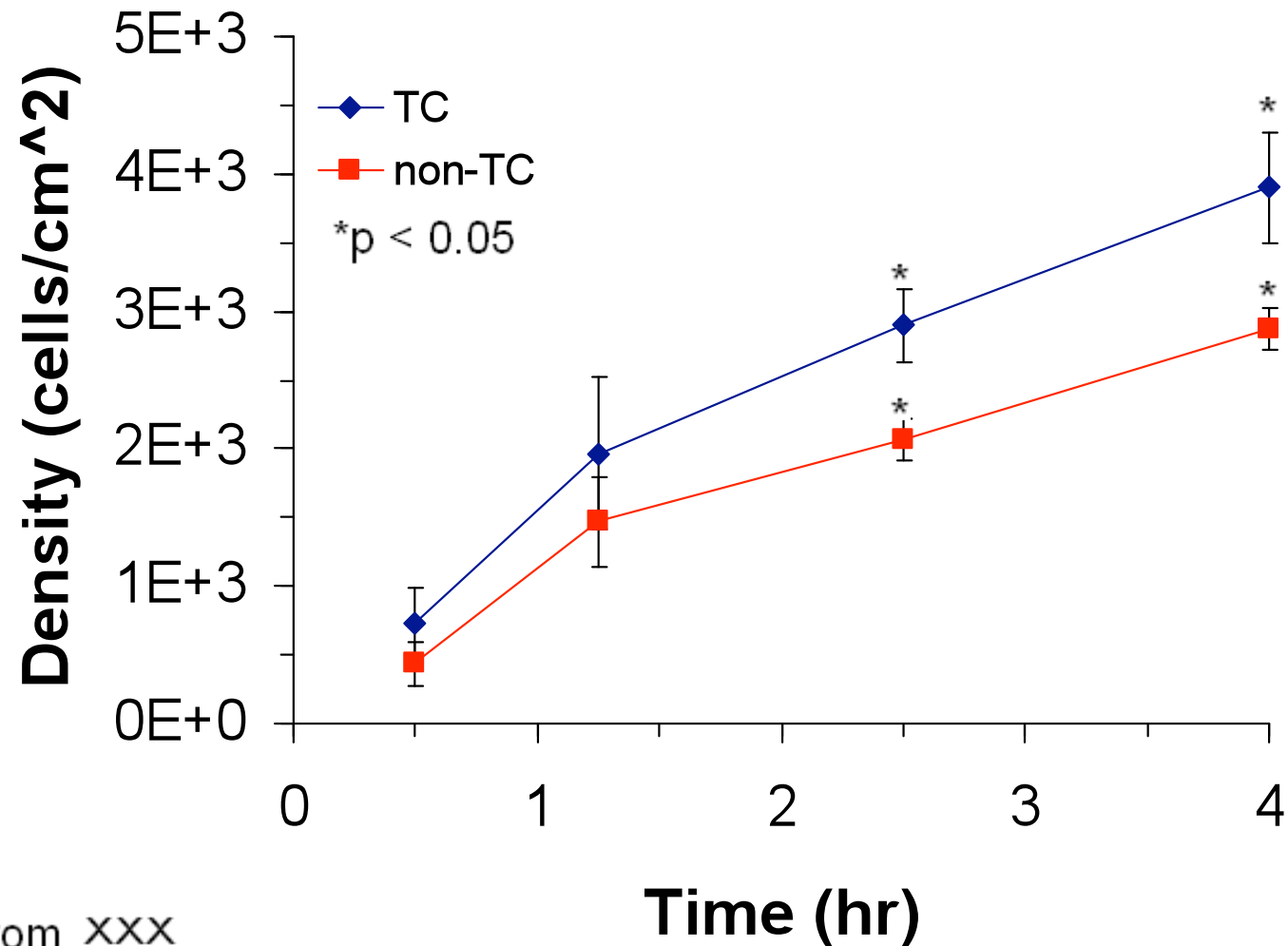
- 24-well plates seeded with $5.0E3$ cells/well
- Cells grown in media containing 1%, 5%, and 10% FBS for 0, 2, 5, and 7 days
- Wells trypsinized and cell number counted using Coulter Counter



Anti-Proliferating Cell Nuclear Antigen (PCNA) Assay Method

- 24-well plates seeded at $2.0E4$ cells/well in media with 1%, 5%, and 10% FBS
- Cells labeled with Anti-PCNA primary antibody
- Bound primary antibody detected with secondary antibody and chromogenic substrate
- Anti-PCNA stained cells identified using light microscopy

Cell Attachment Assay Data¹



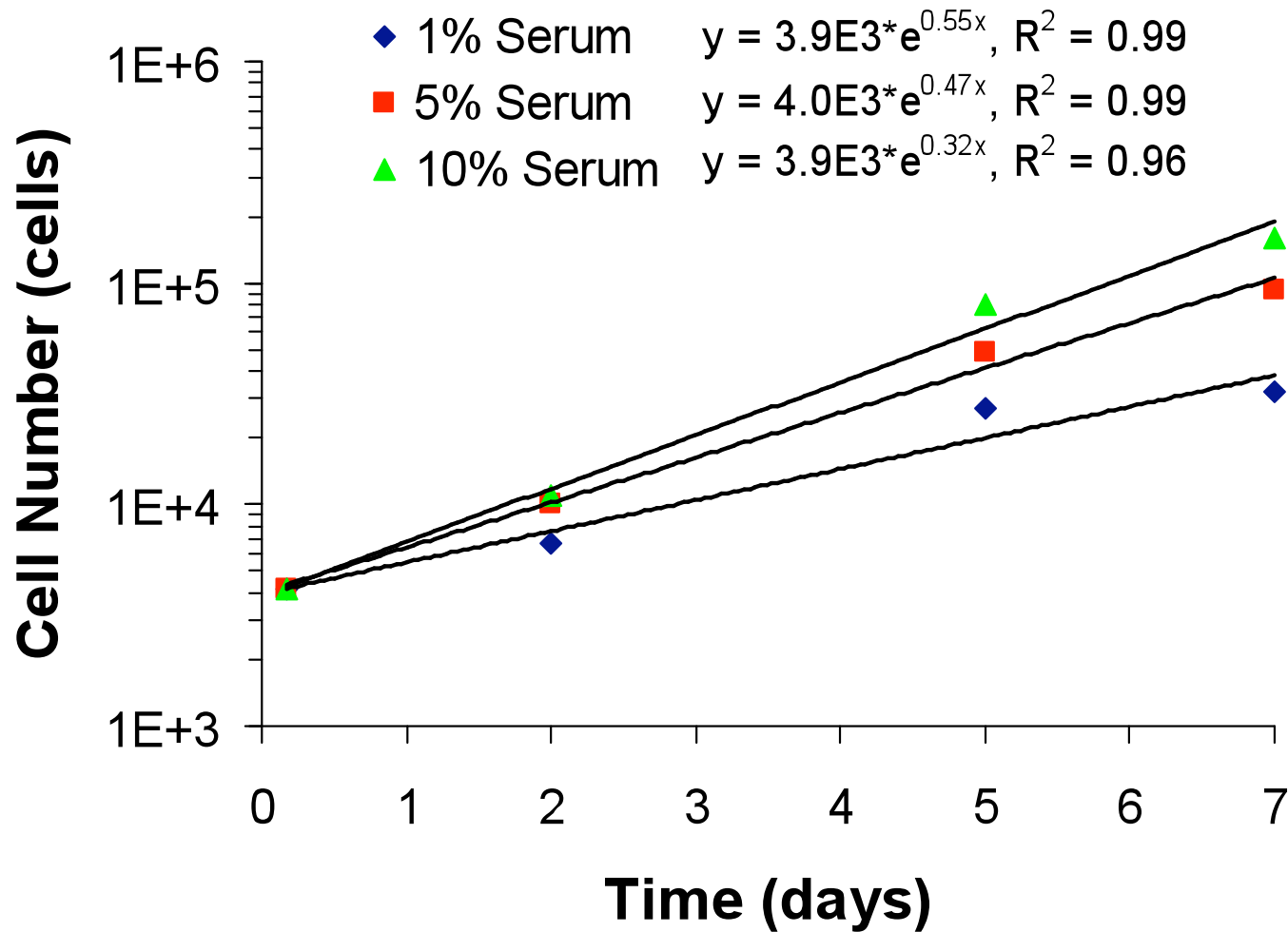
¹Data from XXX



TC Surfaces Promote Attachment

- TC surfaces have higher mean numbers of attached cells compared to non-TC surfaces at all time points
- Cell density on TC vs non-TC surfaces is statistically different for 2.5 and 4 hrs (Student's ttest, $n=3$, $p<0.05$)

Cell Proliferation Assay Data

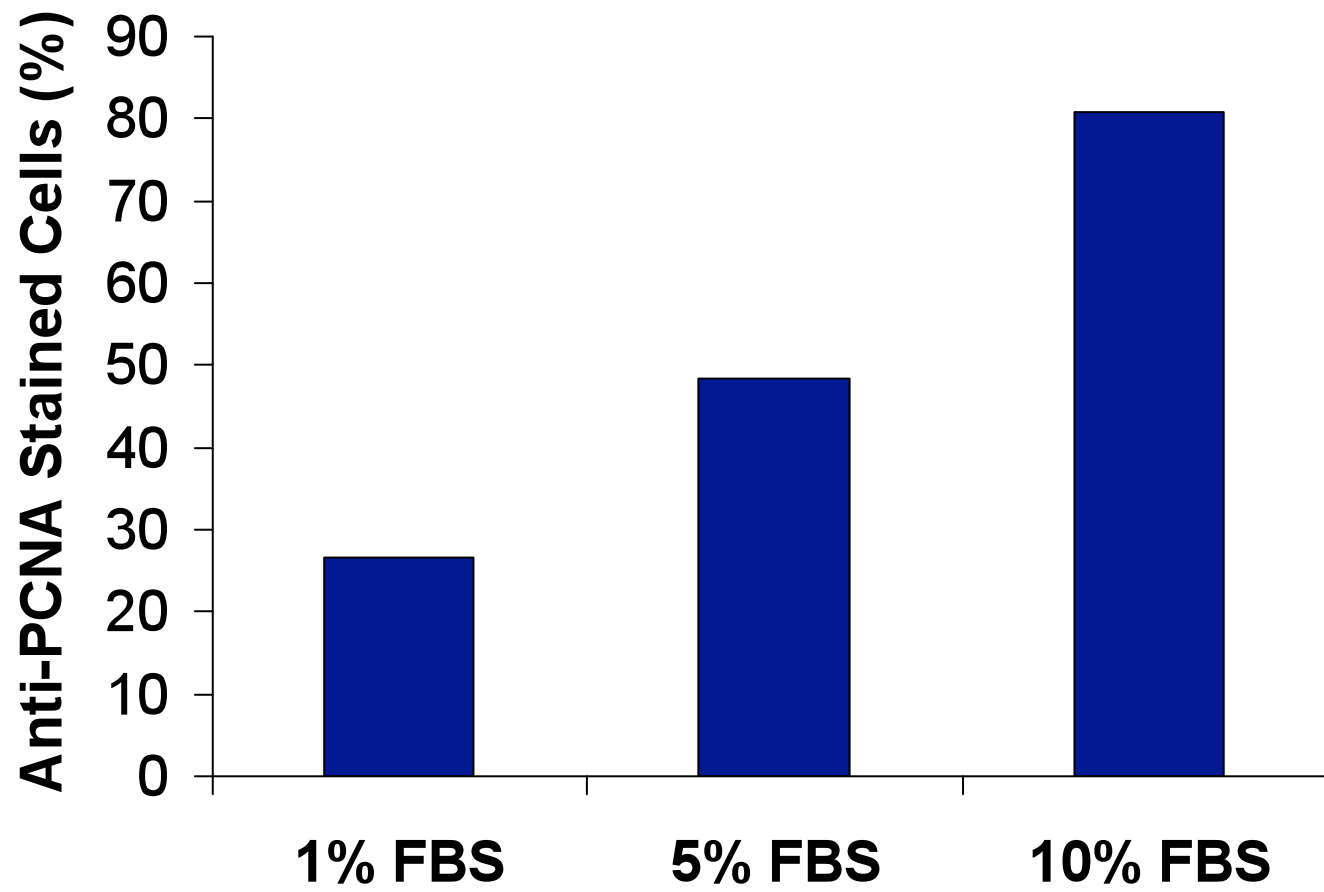




FBS Increases Growth Rate

- Cell numbers of each condition statistically different for Days 2, 5, and 7 (single-factor ANOVA and Tukey's HSD Test, $n=9$, $p<0.05$)
- Growth in all conditions is exponential
- Faster growth rates exhibited in conditions with larger percentages of FBS

Anti-PCNA Assay Data





FBS Increases Fraction of Cells in Synthesis Phase

- Higher fraction of Anti-PCNA labeled cells in conditions with larger percentages of FBS
- Anti-PCNA labels cells in the S-phase



Cell Proliferation and Anti-PCNA Data Are Consistent

- Cells divide soon after passing through S-phase
 - Conditions with large fractions of S-phase cells should correlate with rapid cell growth
- Anti-PCNA Assay determined that fraction of S-phase cells increases with higher FBS percentages
- Cell Proliferation Assay determined increased HDF growth with higher FBS percentages



Summary

- HDF attachment is superior on TC treated surfaces compared to non-TC treated surfaces
- HDF proliferation is faster in higher percentages of FBS.