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# The Effects of Plate Treatment and Serum Level on HDF Attachment and Proliferation

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BIOE 342 – Tissue Culture Laboratory  
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# The Proposed Argument

I Intend To Show:

- Extent of HDF attachment over a 4 hour period is dependent on the type of plate treatment
  - Increases in FBS concentration in HDF growth media increases cell division and cell proliferation
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# Quantitative Attachment Assays

## Measure Cell Attachment Over Time

- HDF cells were seeded in untreated, TC-treated, and Fn-treated 24-well plates
  - Attachment/Cell Density (cells/ml) measurements were taken in triplicate at 30 min, 1.25 hr, 2.5 hr, and 4 hr using Coulter Counters.
  - Data for Untreated and TC-treated from Student XXX
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# Anti-PCNA Staining Stains Nuclei of S-phase HDF Cells Red

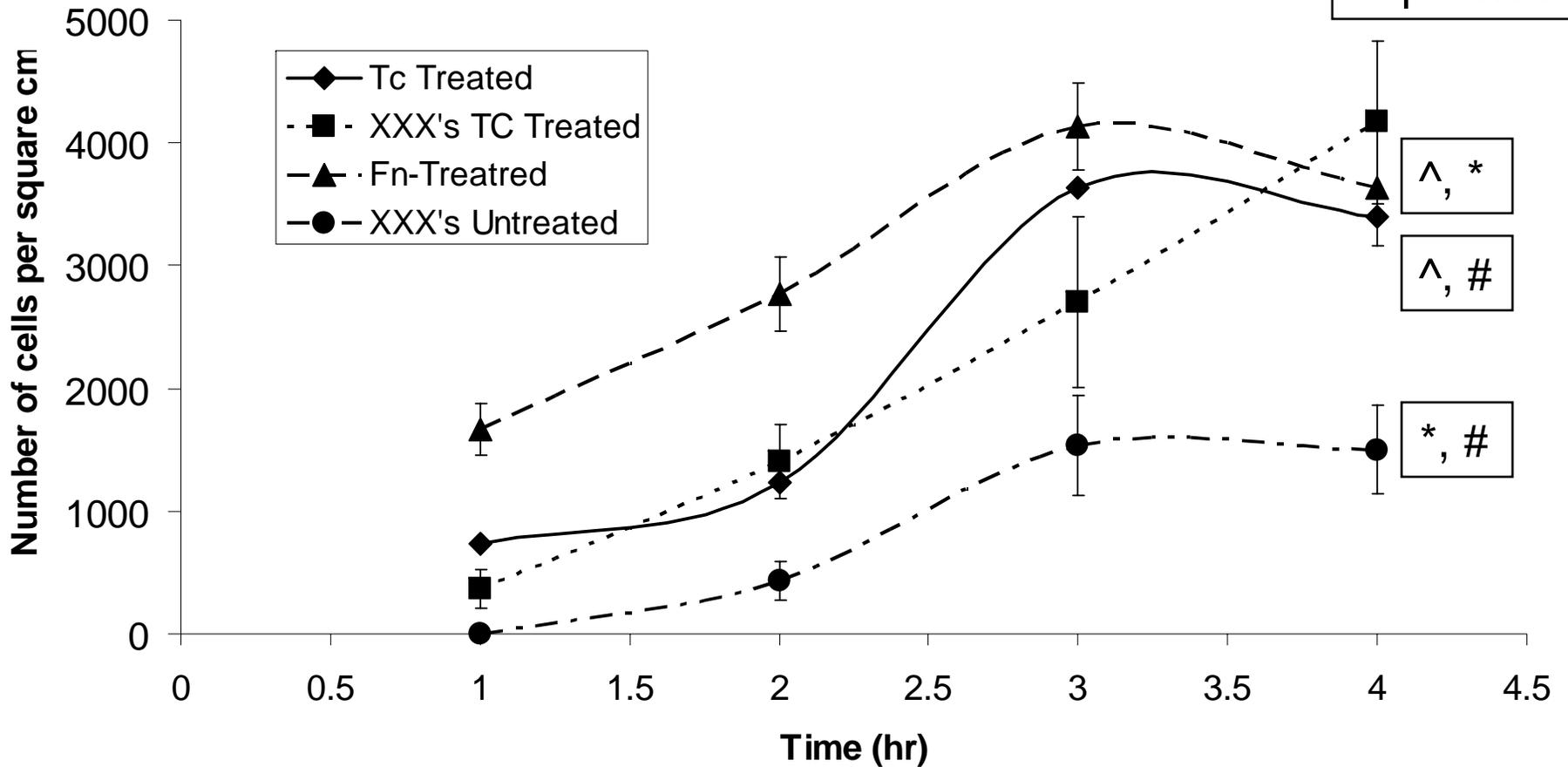
- HDF cells were seeded and grown on 24-well plate under 1%, 5%, or 10% FBS conditions.
  - Cells were treated with Anti-PCNA/Anti-Mouse w/HRP (1°/2°) antibodies coupled with AEC chromogen to stain the S-phase nuclear protein PCNA red.
  - % of cells in S-phase were measured using light microscope
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# Cell Proliferation Assay Measures Extent and Rate of HDF Cell Growth

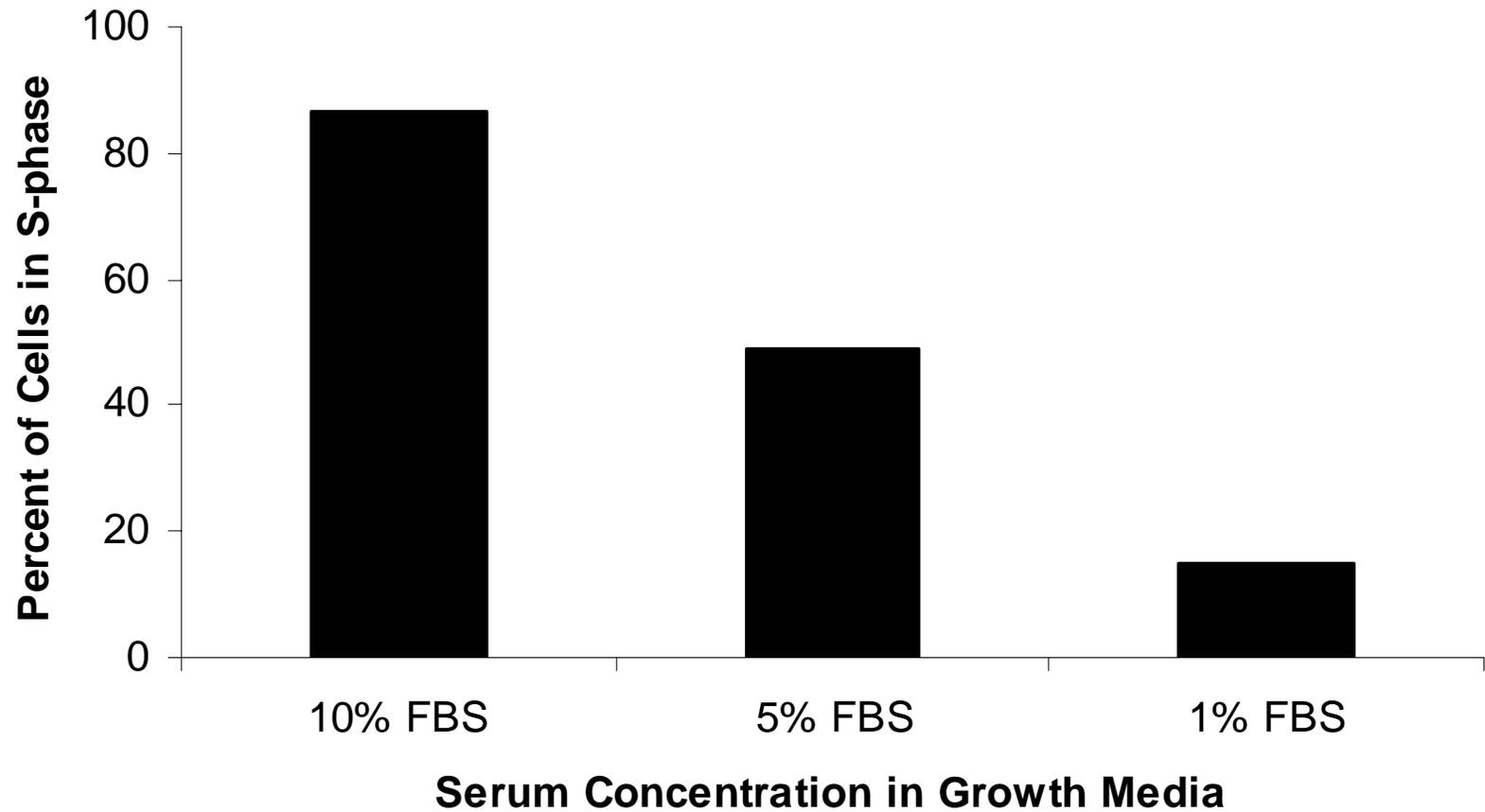
- HDF cells were seeded under one of three media conditions 1%, 5%, or 10% FBS
  - Cell Density (cells/ml) measurements were taken in triplicate at days 0, 2, 5, and 7 using Coulter Counters
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## Different Plate Treatments Yield Different Extents of Attachment

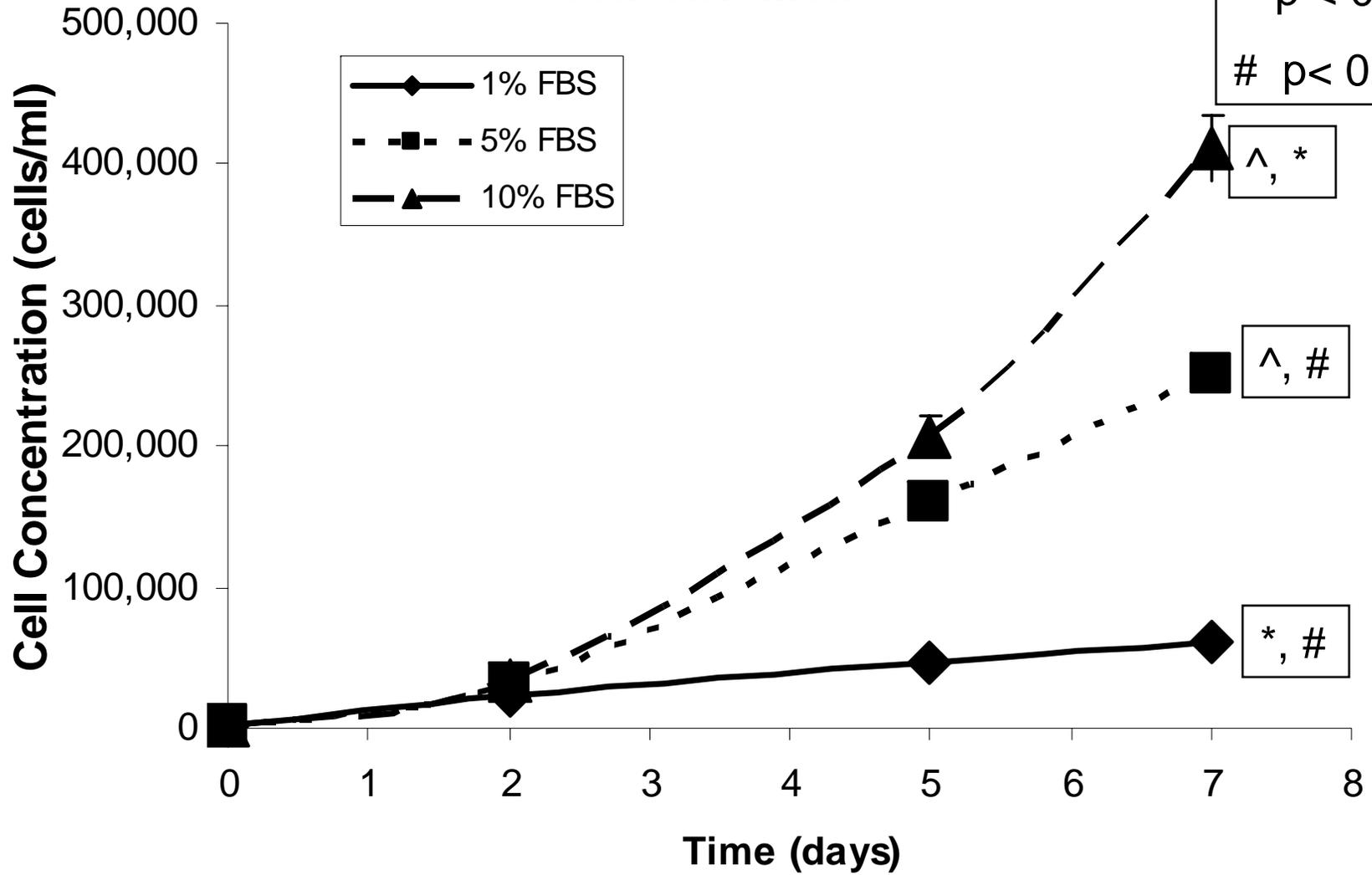


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## As Serum Concentration Increases, So Too Does the Number of HDF Cells in S-Phase



## Proliferation Rate Increases with Serum Concentration



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# Doubling Times During Exponential Growth (Days 0-2 of Cell Proliferation)

1% FBS	5% FBS	10% FBS
0.86 days	0.59 days	0.58 days

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# Plate Treatment Type Affects Cell Attachment

- Significant differences between attachment of HDF cells to treated surfaces as opposed to untreated
  - Probably due to the fact the ECM proteins of cells allow them to better adhere to Fn-treated surfaces
  - The overall negative charge of cells probably allows adherence to TC (charge) surfaces better than untreated
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# Anti-PCNA Staining and Cell Proliferation Assay Agree

- Anti-PCNA staining showed an increase in the number of cells entering S-phase with increased FBS levels
  - Cell Proliferation Assay showed an increase in overall cell growth and doubling time with increases in serum concentration
  - The results suggest that increased serum concentration promotes cell proliferation by increasing the number of HDF cells entering into S-phase and division.
  - Probably because serum contains growth factors that promote cell division
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