HDF Cell Attachment and Proliferation

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Objectives

- To quantitatively measure how choice of culture vessel affects Human Dermal Fibroblast (HDF) attachment
- To analyze the effects of serum concentration on HDF proliferation

Measuring Cell Attachment

- 10,000 HDF cells (P8) in complete media were seeded to 12 wells on 2 TC-treated, 1 untreated and 1 Fibronectin (Fn) coated 24-well polystyrene plates
- After 30 mins, 2 hrs 15 mins, 3 hrs 30 mins and 4 hrs 30 mins three wells on each plate were rinsed thoroughly with Phosphate Buffered Saline (PBS) and cells were visualized with a light microscope. Morphology was noted and cell density was determined by counting the number of cells in a representative 0.01 cm^2 area.

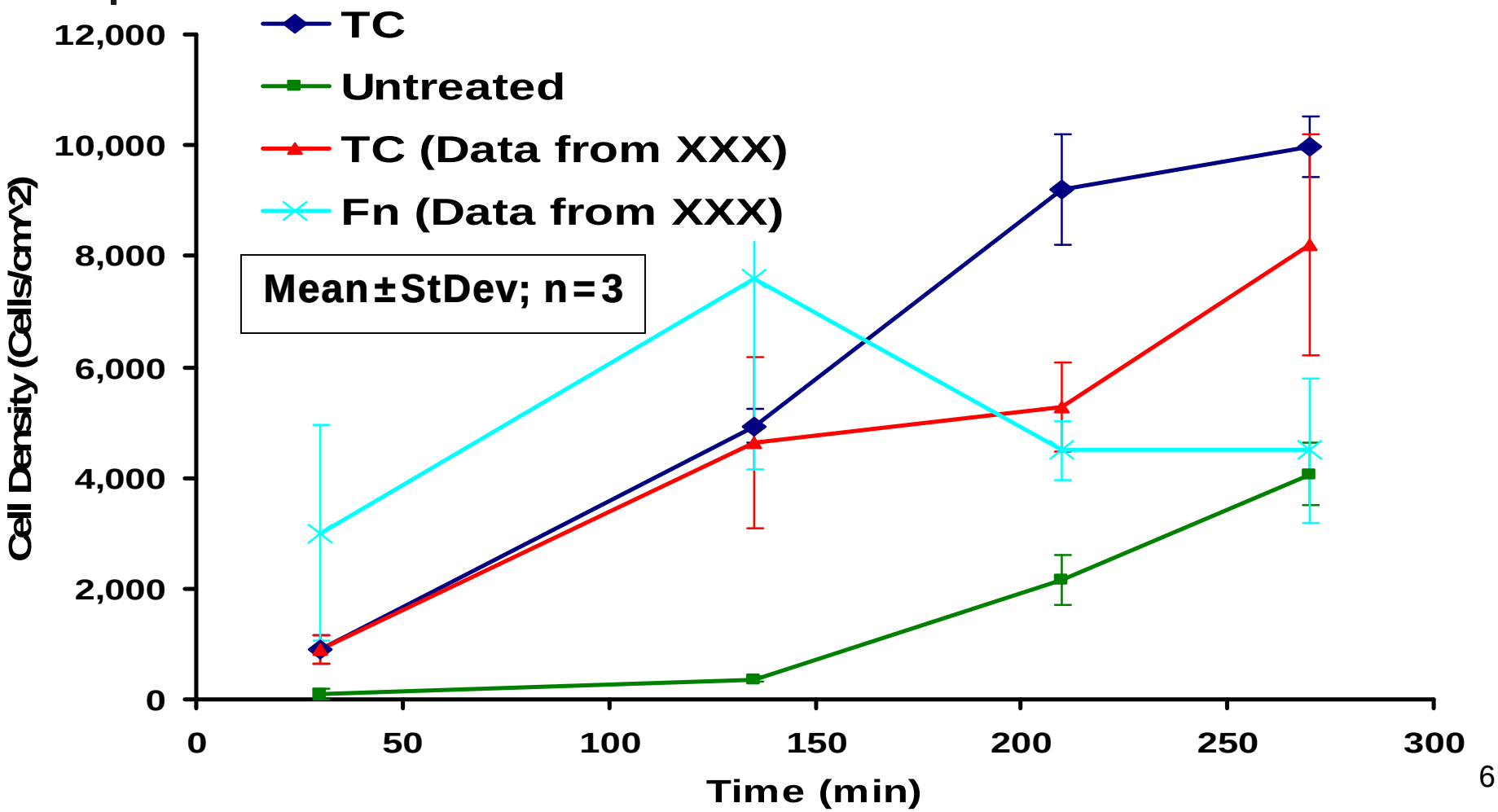
Assessing Cell Proliferation: Anti-PCNA Staining

- 20,000 HDF cells (P8) were seeded to TC-treated wells in DMEM with 1%, 5% and 10% Fetal Bovine Serum (FBS)
- After incubation for 48 hrs, cells were fixed with formalin and stained blue with hematoxylin. Nuclei of cells in S-phase were stained red by anti-PCNA staining.
- Percentage of cells in S-phase was determined for each FBS concentration by performing a total and S-phase cell count in a representative area using a light microscope

Assessing Cell Proliferation: Cell Proliferation Assay

- 5,000 HDF cells (P9) in DMEM with 1% FBS were seeded to wells on 2 TC-treated plates
- After 4 hrs incubation 6 wells were trypsinized and cell number was determined with a Coulter counter. Media was changed in the remaining wells for a total of 9 wells containing 1%, 5% and 10% FBS.
- After 2, 5 and 7 days of incubation 3 wells at each serum concentration were trypsinized and counted using a Coulter counter

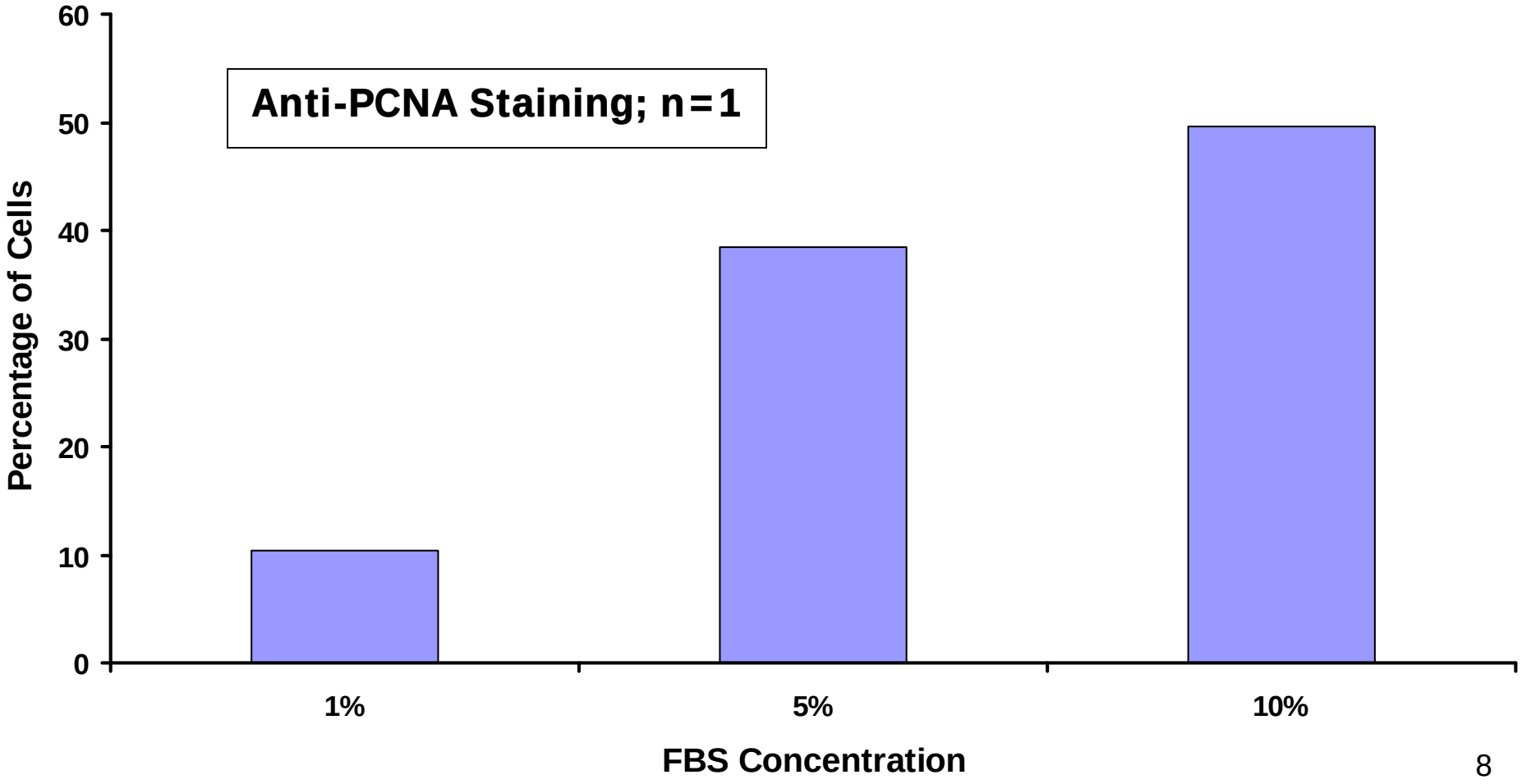
Surface Treatment Affects HDF Attachment



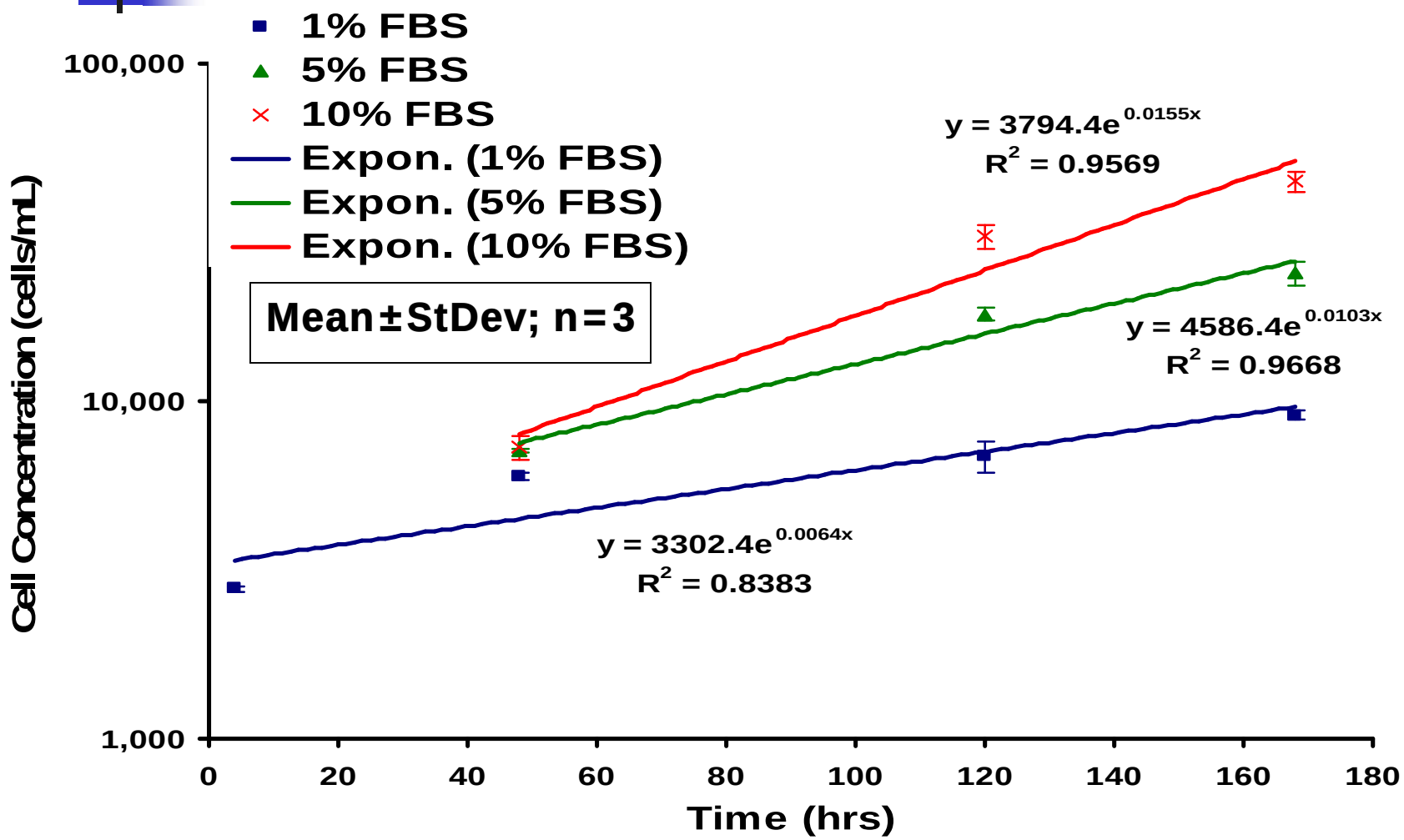
Surface Treatment Affects HDF Attachment and Morphology

- HDF cells attached most slowly to untreated polystyrene and after 4 hrs 30 mins remained relatively small and rounded with few pseudopodia
- HDF cells attached more quickly to TC-treated polystyrene and by 4 hrs 30 mins had become elongated and developed pseudopodia
- HDF cells attached most quickly to Fn-coated polystyrene and by 2 hrs 15 mins had become elongated and developed pseudopodia

FBS Increases Percentage of Cells in S-Phase



HDF Cells Exhibit Exponential Growth



Increasing FBS Concentration Increases Cell Proliferation Rate

- An increase in the percentage of cells in S-phase indicates an increase in the frequency of division
- The Anti-PCNA staining and cell proliferation experiments both demonstrate that increasing the FBS content of the media results in an increase in HDF proliferation rate

Conclusions

- Presence of surface ligands (e.g. Fn, charged moieties) facilitate attachment, elongation and development of pseudopodia for HDF cells
- Growth factors in FBS increase the proliferation rate of HDF cells