Promotion of HDF Cell Attachment and Proliferation

Objectives

- To qualitatively assess the effect of fibronectin (Fn) on HDF cell attachment
  - Fn Attachment Assay
- To observe HDF cell proliferation and position in cell cycle in response to fetal bovine serum (FBS)
  - Anti-PCNA Staining
- To quantitatively evaluate HDF cell proliferation as a result of FBS percentage of media
  - Cell Proliferation Assay

Fn Attachment Assay Methods

- Four conditions of TC-treated wells:
  - A) No Fn in well (control)
  - B) Half of well coated with Fn
  - C) Fn pattern ‘painted’ on surface of well
  - D) Entire well coated with Fn

- HDF cells seeded at 50,000 cells/well and incubated 2 hours at 37°C
- Wells rinsed with PBS to remove unattached cells
- Cell attachment observed with a light microscope

Fn Promotes HDF Attachment

A: No Fn
Very few cells (not spread)
B: Half Fn
Clear boundary between many and very few cells (Fig 1)
C: Pattern of Fn
Cell attachment pattern matches painted Fn pattern (Fig 2)
D: All Fn
Many cells covering well

Note: Sample view fields (illustrations) not to scale nor directly from experiment

Figure 1. Half Fn

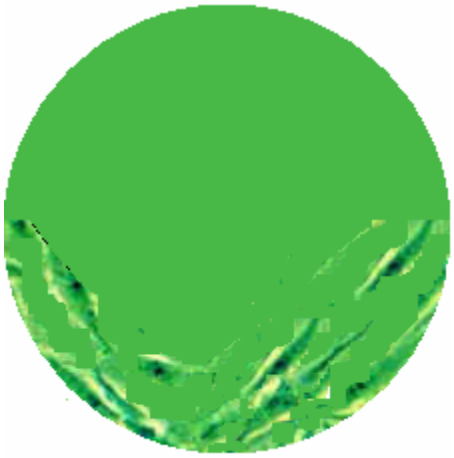


Figure 2. Pattern

Anti-PCNA Methods

- 20,000 HDF cells/well seeded in DMEM with 1%, 5%, or 10% fetal bovine serum (FBS)
- 3 control wells seeded in DMEM with 10% FBS
- Experimental conditions treated with, in order listed: formalin, methanol with 3% H_2O_2, Anti-PCNA primary antibody, Anti-mouse IgG secondary antibody, AEC, and hematoxylin
- Control conditions treated the same except:
  - Control 1: Not treated with secondary antibody
  - Control 2: Not treated with primary antibody
  - Control 3: Not treated with either antibody
- Cells viewed using a light microscope

Serum Affects HDF Cell Cycle

Conditions	% Red Nuclei	Results
1% Serum	45	45% cells in S-phase
5% Serum	80	80% cells in S-phase
10% Serum	70	70% cells in S-phase
Controls	0	Reagents work correctly

Data from XXX

- Anti-PCNA assay stains nuclei in S-phase red
- 5% Serum Condition:
  - Greater percentage of cells in S-phase than in other conditions
  - More cells in S-phase indicates greater involvement in mitosis

Anti-PCNA Observations Suggest That FBS Promotes Proliferation

•10% serum condition: cells proliferated the most (highest confluency)

•5% serum condition: cells have greater involvement in mitosis (highest percentage of red nuclei)

Conditions*	Confluency (%)
1% Serum	40
5% Serum	60
10% Serum	65

*** After 2 days of incubation**

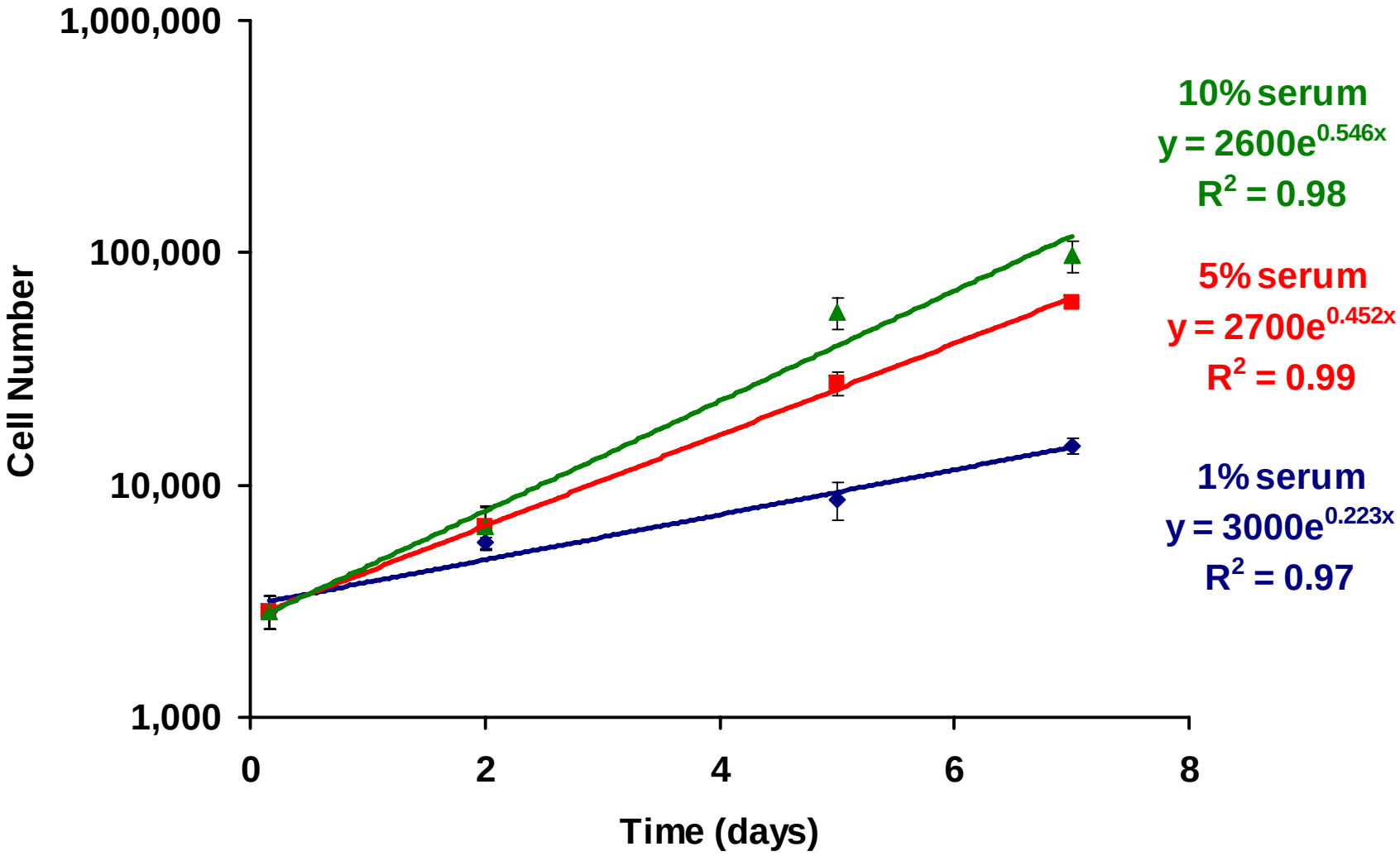
•Because of contact inhibition, 2 days after cell plating:

10% condition (higher confluency) less suitable for proliferation than the 5% condition (lower confluency)

Cell Proliferation Assay Methods

- 5,000 cells/well plated in DMEM with 1% FBS
- After 4 hours incubation:
  - Cell concentration of wells determined with Coulter Counter
  - Cell media changed to 1%, 5%, or 10% FBS
- Cells incubated for 2, 5, or 7 days at 37°C with media replenishment every 2-3 days
- After incubation time:
  - Cell density estimated using light microscope
  - Cell number determined with Coulter Counter

FBS Promotes Cell Proliferation



HDF Cells Have a Positive Growth Response to FBS

- Cell concentrations of the different conditions at 7 days are significantly different (ANOVA, $p<0.0001$).
- More FBS → More HDF cells
  - FBS promoted cell proliferation.
- Cell doubling time (t_D) decreases for an increase in FBS.

FBS %	t_D (days)
1	3.1
5	1.5
10	1.3

- Cells are in exponential growth.
  - Exponential best fit lines of 10%, 5%, and 1% ($R^2 > 0.95$)

Assessment of Serum's Effect on HDF Cell Proliferation

- Both the Anti-PCNA staining and Cell Proliferation Assay demonstrated that serum promotes cell proliferation.
  - HDF confluency for both experiments:
    - greatest for 10% serum
    - least for 1% serum.

- A greater rate of cell proliferation should be correlated with more cells in S-phase.
  - Data not consistent with this relationship
  - Possible explanations:
    - Different seeding densities for each assay
    - Not enough Anti-PCNA data for statistical conclusions

Conclusions

- The Fn Attachment Assay confirmed that Fn encourages HDF cell attachment.
  - Fn may promote attachment because it is an extracellular matrix protein that binds integrins, cell membrane proteins.
- Using Anti-PCNA Staining, dyed cells demonstrated that FBS percentage affects the number of cells in S-phase.
- The Cell Proliferation Assay showed that FBS promotes cell proliferation.
  - FBS may promote growth by providing growth factors to cells.
- Anti-PCNA confluency observations were consistent with the Cell Proliferation Assay results.