

Fibronectin and Serum Promote Adhesion and Proliferation of Fibroblasts Cells (HDF)

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Purpose

- To determine the effects of fibronectin(Fn)-coated surface on HDF cell adhesion
 - Fibronectin Attachment Assay
- To measure the effects of serum concentration in the media on HDF cell proliferation
 - Anti-PCNA Staining
 - Cell Proliferation Assay

Fibronectin Attachment Assay Methods

- Fn diluted to 50 ug/mL with PBS was used to paint 3 patterns shown below by the solid areas in wells of a non-TC-treated 24-well plate.



- The plate was incubated for 30 minutes.
- 50,000 HDF cells were seeded in Fn-coated and non-coated wells and incubated for 2 hours.
- Patterns of cell attachment were observed with a light microscope.

Anti-PCNA Staining Methods

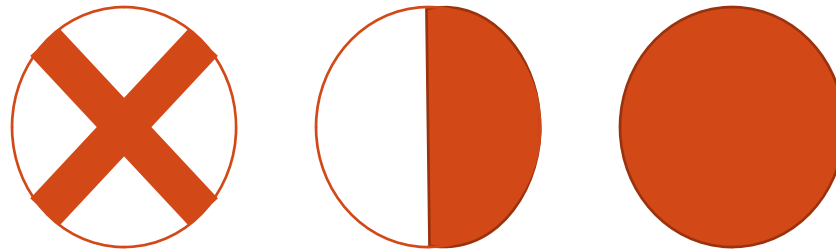
- 20,000 HDF cells in DMEM with 1, 5, or 10% fetal bovine serum (FBS) were seeded into TC-treated 24-well plate and incubated for 2 days.
- Cells were stained with anti-PCAN/Horse Radish Peroxidase (HRP) and Mayer's hematoxylin.
- Under a light microscope, nuclei of cells in S-phase of the cell cycle were stained red by HRP and the others were stained blue by hematoxylin.
- Fractions of cells in S-phase were estimated.

Cell Proliferation Assay

Methods

- 5,000 HDF cells in DMEM with 1%, 5%, or 10% FBS were seeded into TC-treated 24-well plates and incubated.
- At 4 hours, 2, 5, and 7 days of incubation, cell concentration in each condition was determined using a Coulter Counter.

Fibronectin Improves Cell Attachment



- Elongated shaped HDF cells occupy the solid areas show above.
- Patterns of cell attachment closely resemble Fn patterns painted.

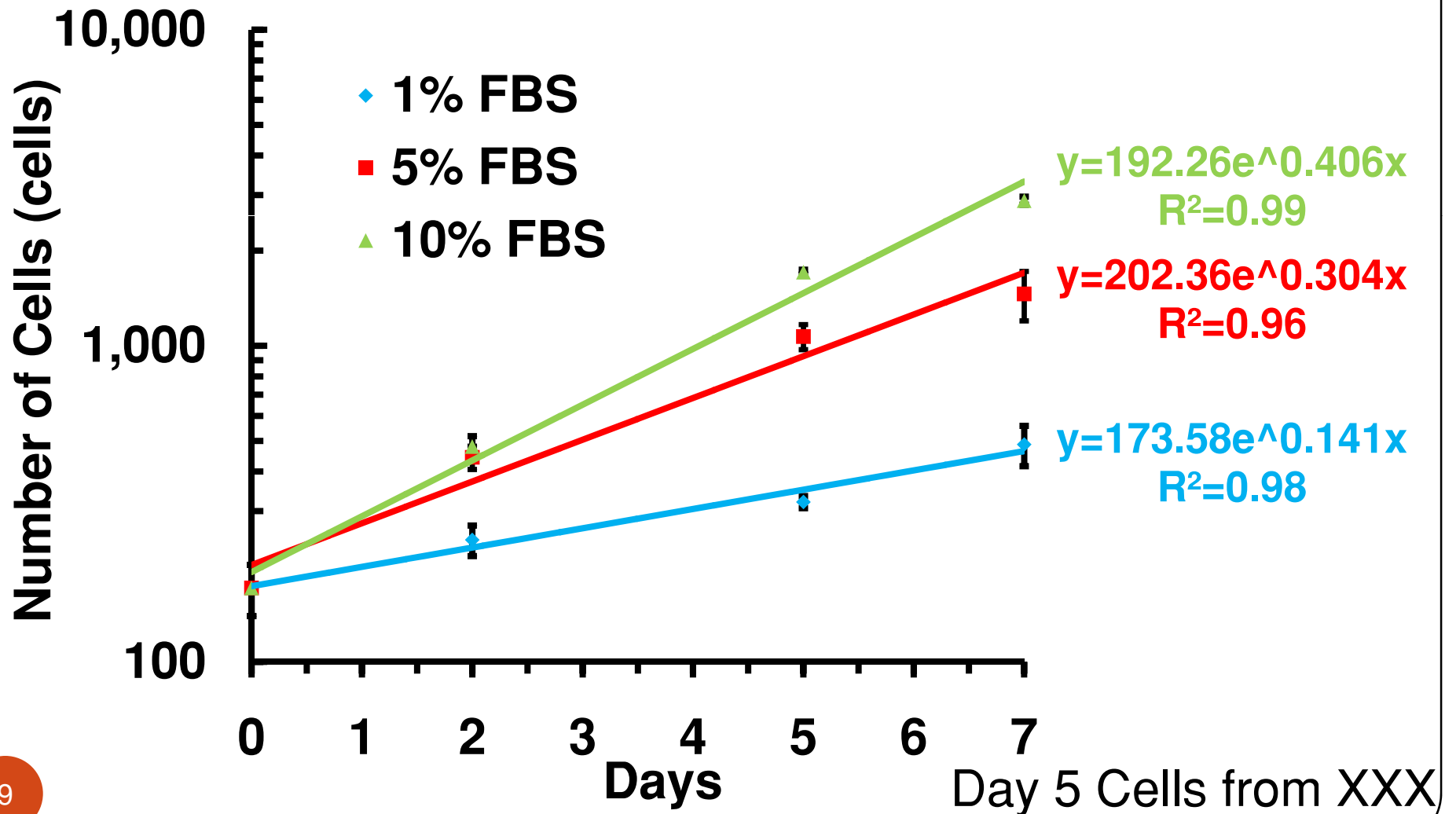
Fraction of HDF in S-phase Increases with FBS Concentration

FBS Concentration (%)	Cells in S-phase (%)	Cells not in S-phase (%)
1	35	65
5	50	50
10	30-80	70-20

Fraction of HDF in S-phase Increases with FBS Concentration

- Fraction of HDF nuclei stained red increases with FBS concentration.
- Fraction of cells in S-phase increases with cell proliferation rate.
- HDF proliferation rate decreases in areas of high cell confluency.

HDF Population Growth Rate Increases with FBS Concentration



HDF Doubling Time Decrease with FBS Concentration

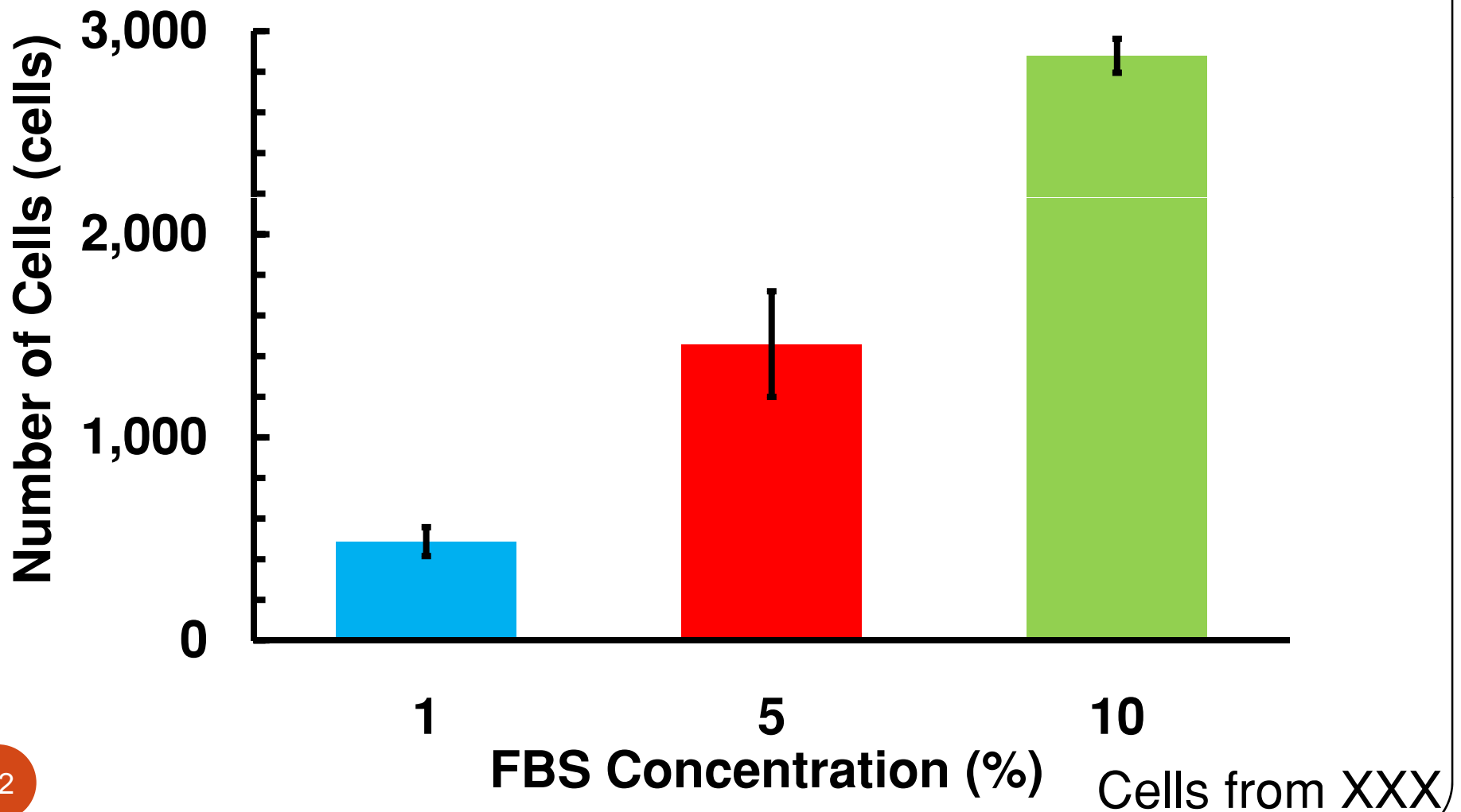
FBS Concentration (%)	Doubling Time (days)
1	4.9
5	2.3
10	1.7

- HDF growth rates in 1, 5, and 10% FBS are statistically different from one another (ANOVA, $p < 0.05$).
- HDF in 10% FBS have shortest doubling time and largest population growth rate.

Significantly Differences in Cell Numbers on Days 2, 5, and 7

- Cell number is larger at Day 5 than Day 2 (paired t-test, $p < 0.001$).
- Cell numbers is larger at Day 7 than Day 5 (paired t-test, $p < 0.002$).
- Cell numbers is larger at Day 7 than Day 2 (paired t-test, $p < 0.001$).

Number of Cells on Day 7 Increases with FBS Concentration



Number of Cells on Day 7 Increases with FBS Concentration

FBS Concentration (%)	1	5	10
Mean Number of Cells (cells)	487	1459	2879

- Number of cells exposed to 1, 5, 10% FBS on Day 7 are statistically different from one another (ANOVA, $p < 0.04$)

Consistent Results of Anti-PCNA Assay and Cell Proliferation Assay

- Population growth rate is a function of number of cells in S-phase in the population.
 - Anti-PCNA Staining assay suggests HDF in media with 10% FBS have higher fraction cells in S-phase than 1 or 5%.
 - Cell Proliferation assay suggests HDF in media with 10% FBS have higher population growth rate than 1 or 5%.

Effects of Fibronectin and BFS on HDF Adhesion and Proliferation

- Fibronectin-coated surface promotes HDF cell attachment in *vitro*.
- HDF population growth rate in media with 10% BFS is higher than that in media with 1 or 5% BSF in *vitro*.
- Whether population growth rate increases with serum concentration above 10% need to be tested.