Accessing Fibroblast Proliferation, Function, and Toxicity using Different Biological Assays

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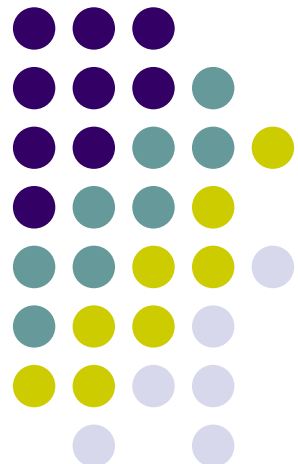
Feb 11, 2009

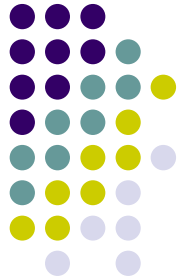


Objectives

- Is fibroblast proliferation and function affected by different serum concentrations in media?

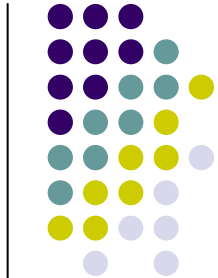
- How can cell toxicity be assessed using fluorescence microscope?

Cell Proliferation Assay:
Counting cell number over time



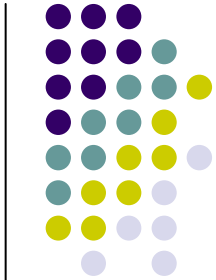
- 5,000 cells/mL were prepared and seeded in 24 well TC- treated plates
- 1%, 5%, and 10% serum media was added for every 3 wells.
- Cells were counted using Coulter Counter at 4hrs, 2 days, 5 days, and 7 days after seeding

Anti-PCNA Staining Assay:
Visual Assessment of Cell Proliferation



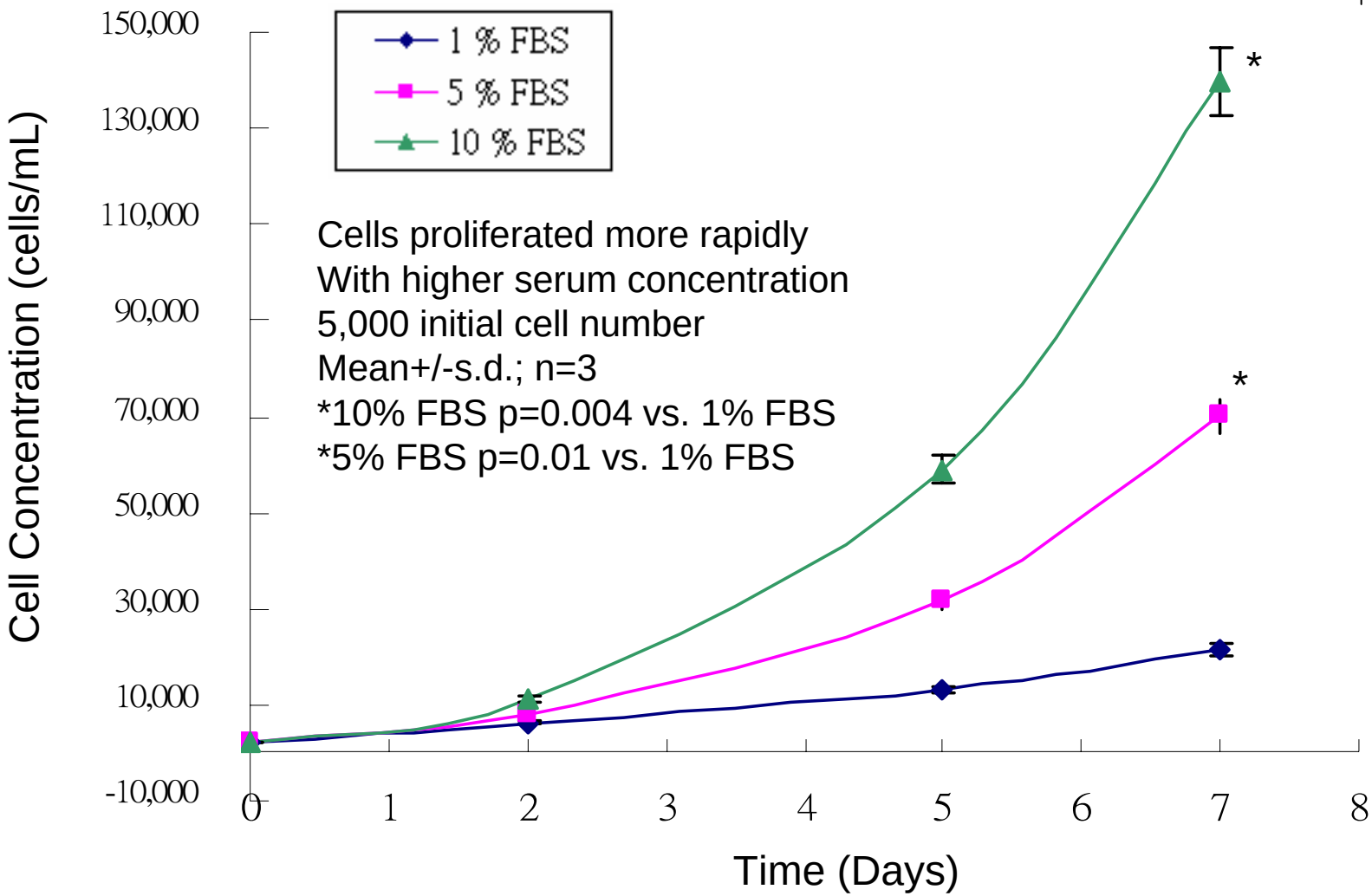
- 20,000 cells/mL were seeded in media with 1%, 5%, and 10% serum and incubated for 2 days. Controls set up with 10% serum
- Blocking buffer used to allow Anti-PCNA primary antibody and Anti-mouse IgG secondary antibody attachment.
- AEC solution, hematoxylin, and NH4OH stains were added to complete Anti-PCNA assay
- PCNA stained cells were observed under light microscope to access cell function

Live/Dead Fluorescence Assay:
Observing cell viability using fluorescence

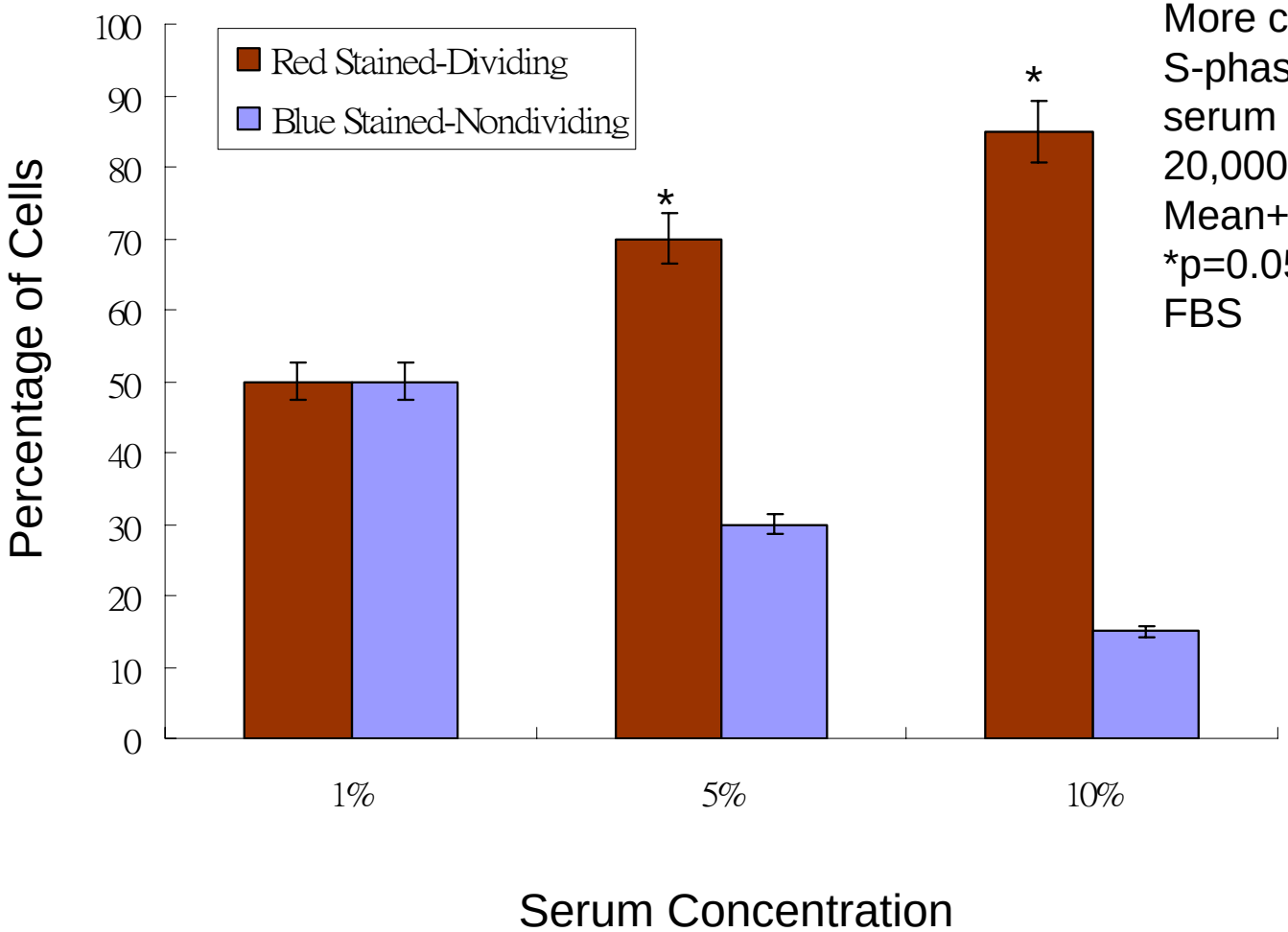


- 90% confluent cells diluted in media with 1:3 ratio
- Diluted cells seeded in TC-treated plates in media with 10% serum and 1% antibiotic
- Seeded cells incubated for 2 nights
- After incubation, cells were rinsed with PBS
- Fluorescent dye and different amounts of ethanol were added
- Cells were observed under fluorescent microscope to access toxicity

Serum Concentration Increases Cell number



Serum concentration increases percentage of cells in S-phase



More cells in S-phase with higher serum concentration
20,000 initial cell number
Mean+/-s.d.; n=1
*p=0.05 and 0.08 vs. 1% FBS

Observations for Live/Dead Assay Under Fluorescence Microscope

- Control cells with fluorescence dye were stained green
- Cells with ethanol were stained red in nucleus, indicating cell death
- Cells with 2 drops of ethanol showed low amounts of red stain among green stained cells

Fibroblast Response for Cell Proliferation Assays

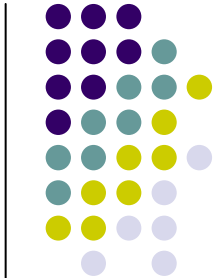
- Cell Proliferation Assay

- Cell number increased with increasing amounts of serum concentration in media

- Anti-PCNA Staining

- Percentage of cells undergoing S-phase increased with serum concentration increase

Fibroblast Response for Cell Toxicity Assay



- Live/Dead Fluorescence Assay
- Cells tagged with fluorescence protein showed toxicity of alcohol by florescence difference
- Fluorescence microscope advantage: Provides immediate visual recognition of live or dead cells.