Characterizing how *in vitro* media conditions affect the proliferation and viability of human dermal fibroblasts

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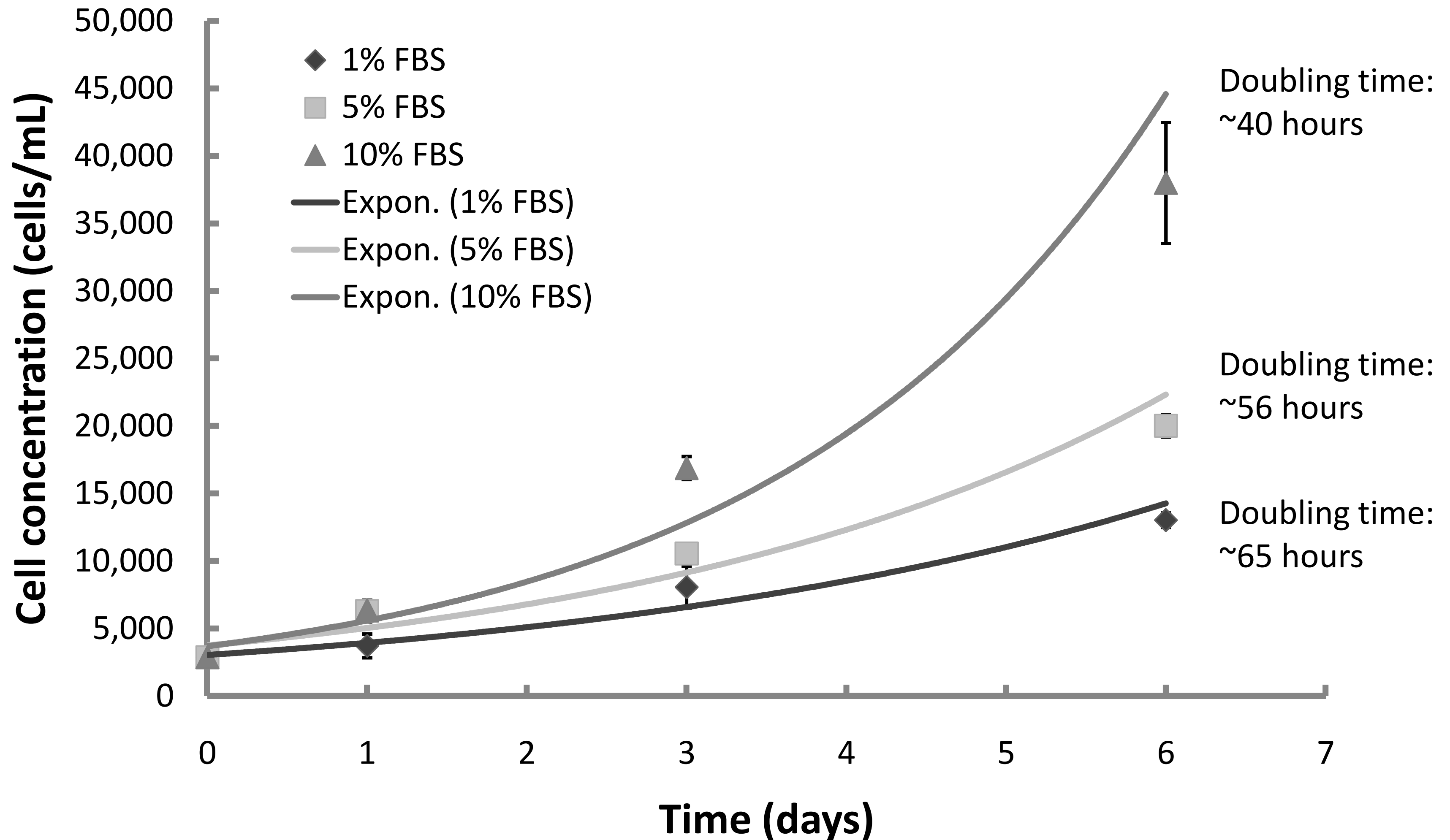
Objectives

- To determine how Fetal bovine serum (FBS) affects the proliferation of Human dermal fibroblasts (HDF)
  - Does the % FBS affect the doubling time of HDF cells? (Proliferation assay)
  - Does the amount of FBS affect the % of cells dividing at a specific time point? (Anti-PCNA staining assay)
- To characterize how ethanol concentration affects human dermal fibroblast viability
  - Are high levels of ethanol are rapidly fatal to HDF cells? (Live/Dead assay)

Proliferation Assay: correlating the proliferation rate with the % FBS

- HDF cells were grown in DMEM with three different percentages of FBS: 1%, 5%, and 10%.
- Cells were counted at 4 time points (Day 0, 1, 3, and 6) to determine how much proliferation had occurred.
  - Cell counting was performed using a Coulter Counter. All measurements were taken in triplicate.
- The media was changed every few days to ensure no significant depletion of nutrients or buildup of wastes.

HDF proliferation rate appears to increase with the % FBS in the media



A statistical analysis shows that this effect is significant

- After 6 days of growth, the HDF cells reached different concentrations:
  - 1% FBS: 13,000 ± 537 cells/mL
  - 5% FBS: 20,000 ± 866 cells/mL
  - 10% FBS: 38,000 ± 4,480 cells/mL
- The 10% FBS cells grew to a significantly higher concentration than either the 5% or 1% FBS cells ($p<0.05$ using a univariate ANOVA with post-hoc Tukey's test)
  - However, there was no statistical difference between the final concentrations of the 1% and 5% FBS test groups ($p>0.05$)

Anti-PCNA Staining: determining how the % of dividing HDF cells is affected by FBS levels

- HDF cells were grown in DMEM with three different percentages of FBS: 1%, 5%, and 10%.
- After 2 days of growth, these cells were fixed and stained using hematoxylin and an anti-Proliferating Cell Nuclear Antigen (PCNA) immunohistochemical stain
- The cells were visualized under a light microscope, which allowed determination of the percentage of cells currently in S-phase
  - Blueish all over = not in S-phase, red nucleus = in S-phase

More FBS causes a higher % of HDF cells to be dividing at a given time

Percentage of FBS in media	Percentage of cells in S-phase
1%	27%
5%	43%
10%	71%

- As the % FBS increases, so does the percentage of cells in S-phase.
  - The highest % FBS tested was 10%, so we do not know if this trend continues at higher amounts of FBS
  - Since only one data point at each % was collected, statistical analysis is not relevant.

Live/Dead Assay: determining how ethanol affects HDF viability

- HDF cells were grown in TC-treated plates for two days, then subjected to different ethanol conditions
  - Group 1: phosphate buffered saline (PBS) added to each well (control)
  - Group 2: 70% ethanol added to each well
  - Group 3: PBS added to each well, then two drops of 70% ethanol added to that
- The cells were stained with ethidium and calcein AM and incubated for 30 mins at room temperature
  - Observations were made with a fluorescent microscope which allowed the detection of living and dead cells due to the dyes

Ethanol appears to kill HDF cells extremely rapidly

Test Conditions	**Observations**
PBS added	All of the cells stained fluorescent green, indicating that they were alive.
70% ethanol added	All of the cell nuclei stained fluorescent red, indicating that they were dead.
PBS added, then two drops of 70% ethanol	Most of the cells on the plate stained green, indicating life. However, there were patched of missing cells ringed by dead cells indicating that localized cell death occurred.

- The patches of dead cells caused by the drops of ethanol indicate that it killed cells <u>before</u> it diffused into the PBS
  - This suggests that ethanol kills HDF cells very quickly
  - Even though the final concentration of ethanol was sub-toxic, many cells died.

The proliferation and anti-PCNA assays show the effect of FBS in two ways

- The proliferation assay and PCNA assay test the same independent variable at the same values (% FBS in media, at 1%, 5%, and 10%).
- Both assays indicate that proliferation is encouraged by higher % FBS, though the results are not quantitatively comparable
  - The proliferation assay shows that the proliferation rate goes up with increasing % FBS
  - The anti-PCNA stain provides a 'snapshot' of cell division, showing that more cells are dividing with higher % FBS
- Additionally, since the highest % FBS tested was 10%, we do not know if this trend will continue as the amount of FBS in the media is raised further.

The composition of the media in HDF culture affects cell proliferation and viability

- When culturing HDF cells *in vitro*, the media composition is an important variable to consider.
  - The assays involving FBS indicate that the exact composition of the media can have a significant long term effect on HDF growth.
  - The Live/Dead assay showed that even the addition of ‘safe’ amounts of ethanol can be harmful to cells because death can occur before the poison fully diffuses to sub-toxic levels.