Viability and Attachment of Human Dermal Fibroblasts

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BIOE 342
Rice University

Objectives: Observing Cell Viability and Cell Proliferation

- Anti-PCNA Staining: Differentiates between human dermal fibroblast (HDF) cells in the S phase and cells in other phases of cell division
  - Detects presence of PCNA, which peaks during S phase
  - Shows relationship between percent cells in S phase and cell proliferation
- MTT Viability Test: Mitochondrial activity in viable HDF cells reduces MTT dye to stain purple.
  - Shows relationship between cell concentration and absorbance
- HDF attachment to different surfaces quantified
  - Conditions: TC-treated, non-TC-treated, and

Methods: Anti-PCNA Staining

- 1mL of HDF cells suspended in DMEM with 1%, 5%, and 10% fetal bovine serum (FBS) incubated for two days
  - Cells treated with formalin, methanol, H_2O_2, and blocking buffer
- 1° antibody applied followed by 45 minute incubation and PBS washes
  - Repeat with 2° antibody
- Cells dyed with AEC and hemotoxylin
  - AEC dyes red – cells in S phase; hemotoxylin dyes blue – other cells
- Cells viewed under light microscope
  - Percent red cells estimated

Methods: MTT Viability Test

- Six cell concentrations incubated for 2 days
  - Two 24-well plates each had 0.5mL of cells in the following concentrations: 50,000, 33,500, 25,000, 16,700, 8,330, and 4,170 $\frac{cells}{mL}$
- Trypsinized and counted each condition in one plate with Coulter counter
- Applied MTT dye to cells in other plate and incubated for 2 hr
  - Following incubuation, applied Solubilization/Stop solution and incubated for 45 min
- Measured and recorded absorbance of each condition with spectrophotometer at 570nm

Methods: Cell Attachment Assay

- Seeded cells in 4 plates
  - Two TC-treated plates, one Fn coated plate, one non-TC-treated plate
- Cells counted with a light microscope 30 min after incubation
  - Microscope set to 10x magnification
  - Only cells in 10x10 grid ($1mm^2$) were counted
- Repeated count at 1 hr 15 min, 2 hr 20 min, and 4 hr after incubation

Methods: Cell Proliferation Assay

- Seeded 1mL of 5,000 cell/mL suspension in 24-well plates
  - Three media conditions: DMEM with 1%, 5%, and 10% FBS
- Seeded each condition in 9 different wells; Seeded an additional 6 wells in 1% FBS
- Cells at each media condition trypsinized and counted with Coulter counter on days 1, 3, and 6
  - Cells in 1% FBS also counted on day 1

Cell Proliferation: Observed with Anti-PCNA Staining and Cell Proliferation Assay

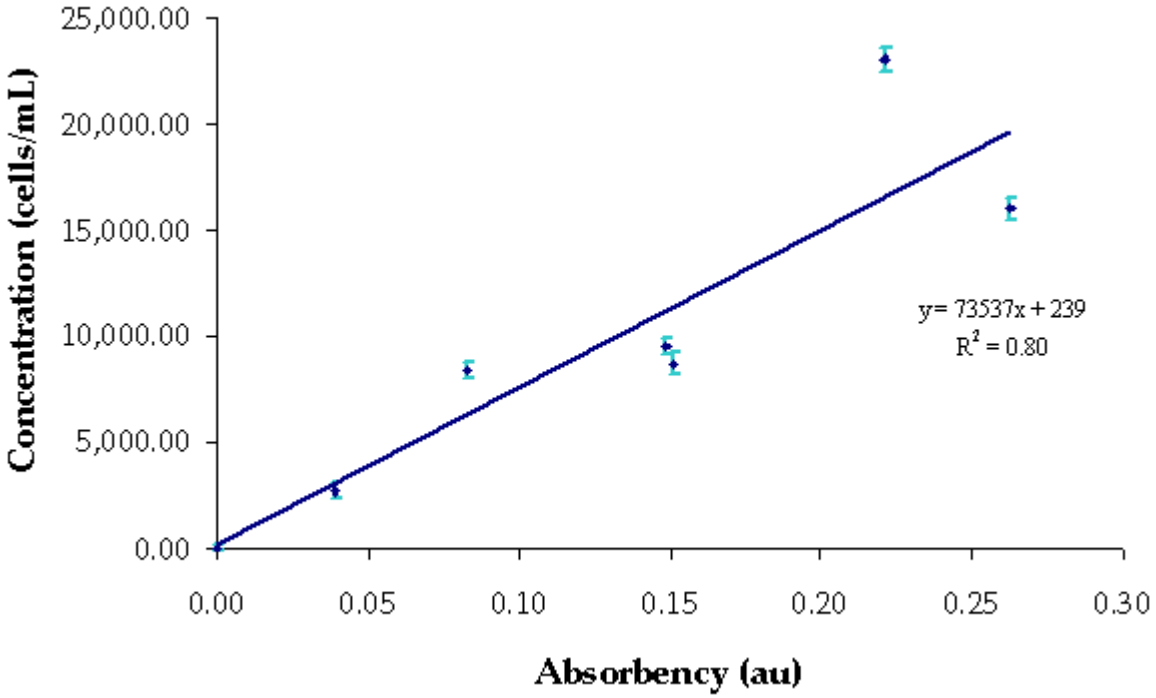
- Cell proliferation is measured in both assays but characterized differently.
  - Percentage of cells in S phase indicate the percentage of cells that will divide and proliferate.
  - Cell concentrations indicate cell growth rate.
- Anti-PCNA Staining does not provide information on cell growth rates.
- Cell proliferation data does not provide information on cell division.

Results: Cells Incubated in DMEM with 10% FBS Contain the Highest Percentage of Cells in S Phase

Media Treatment	Percent Cells in S Phase
1% FBS	50%
5% FBS	65%
10% FBS	85%

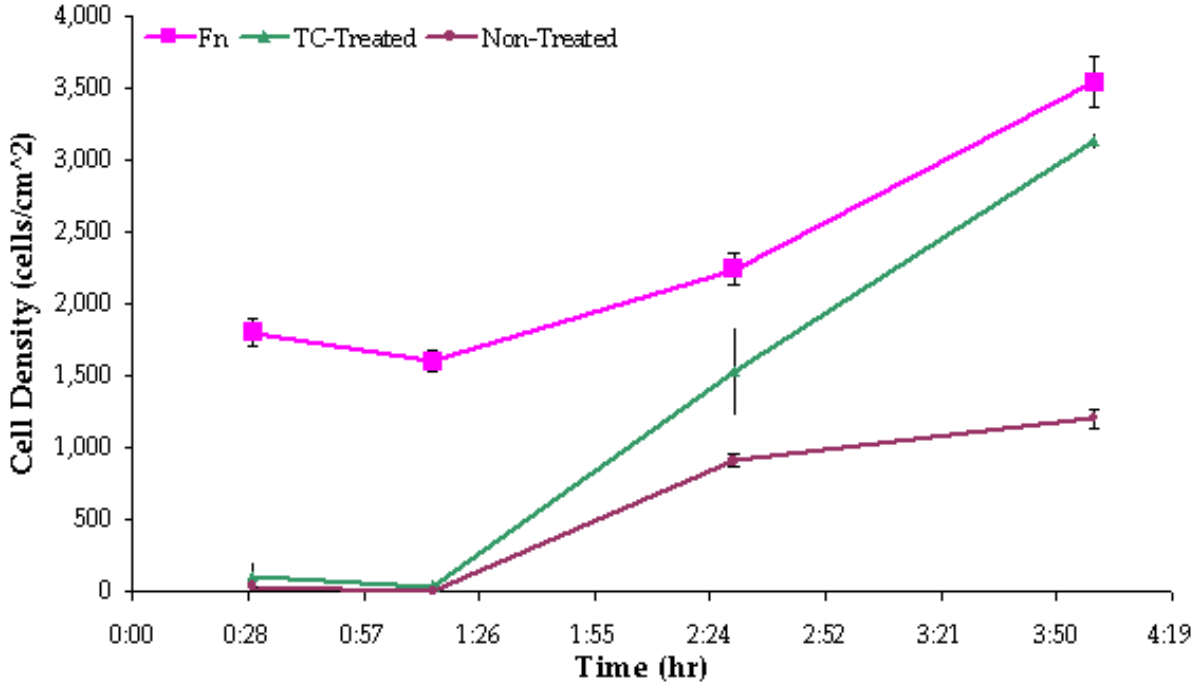
- HDF cells grown in media more rich in nutrients have a higher percentage of cells in S phase.
  - Can be demonstrated for DMEM with 1%, 5%, and 10% FBS.
  - Cells proliferate as a function of available nutrients in surroundings
- Cells in S phase of division had stained red nuclei vs other cells with stained blue

Results: Absorbency Positively Correlates with Concentration



- The absorbency and the concentration of a given solution are positively correlated ($R^2 = 0.80$).
- MTT dyes only viable cells, resulting in a concentration of only viable cells in a culture.

Results: More HDF Cells Attach to Fn Surfaces than to Other Tested Surfaces in 4 Hours



- HDF cells preferentially attach to Fn plates plates.
  - However, cells will still attach and grow on non-treated plates (non-treated data from YYY).
- T-tests conducted between Fn and TC-treated and non-treated and TC-treated data show significantly different densities ($p<0.05$).

Conclusion: HDF Cells Attach and Proliferate Depending on Media and Surface Conditions

- HDF cells grow quickest in media in high concentrations as in anti-PCNA assay and cell proliferation assay
  - More available nutrients allow cells to proliferate faster
  - Cells do not overcrowd areas with low serum concentrations
- Cell viability can be measured with MTT assay.
  - Only viable cells are stained.
  - Assay can be used to determine toxicity in various compounds added to media.
- Cells attach preferentially to Fn coated surfaces.
  - Cells will also grow in untreated surfaces.