

Effect of Culture Conditions on HDF Cell Proliferation and Attachment



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Objectives

- Test differing culture conditions to determine:
 - Optimal seeding surface for maximizing HDF cell attachment
 - Quantitative Cell Attachment Assay
 - Optimal media serum concentration for HDF cell proliferation
 - Anti-PCNA Staining
 - Cell Proliferation Assay



Cell Attachment Quantification

- Cells seeded onto 2 surface conditions
 - TC-treated polystyrene plates
 - Untreated polystyrene plates
- Attached cells in 0.01 cm^2 representative areas counted using light microscope at 4 time points



Cell Proliferation Quantification

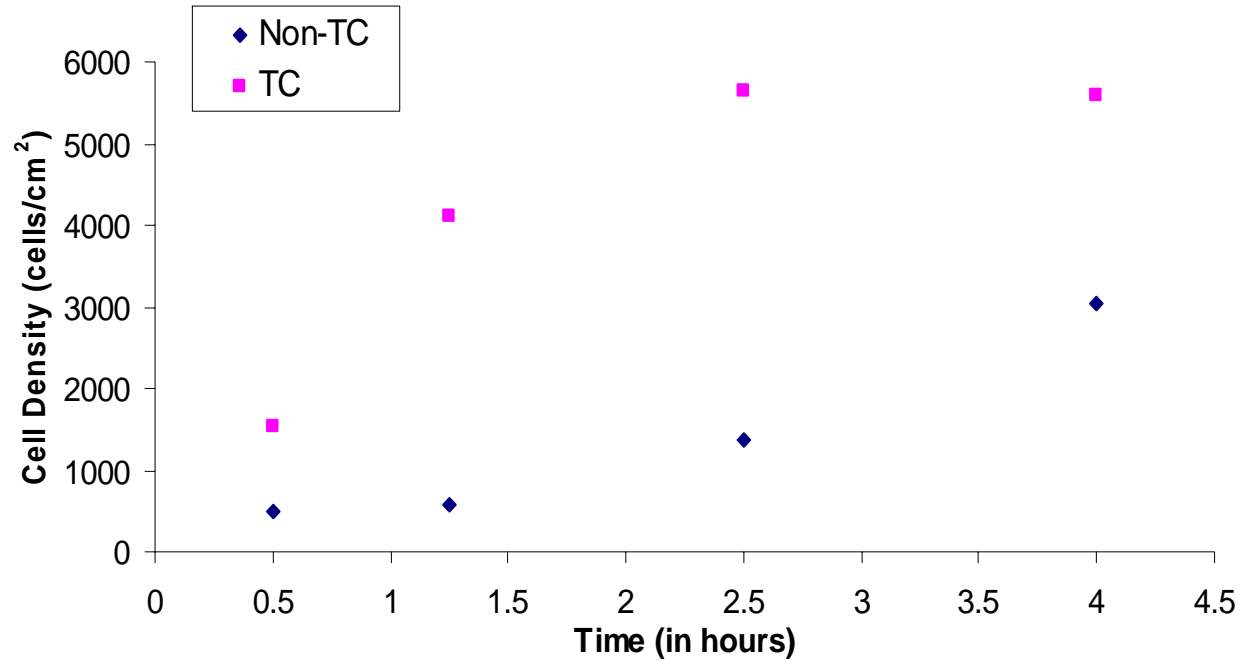
- Cell Proliferation Assay
 - Equal concentration of HDF cells cultured in 3 different concentrations of serum
 - DMEM 1% FBS
 - DMEM 5% FBS
 - DMEM 10% FBS
 - At 4 time points, cells were trypsinized, and the number of attached cells determined using the Coulter Counter



Cell Proliferation Quantification

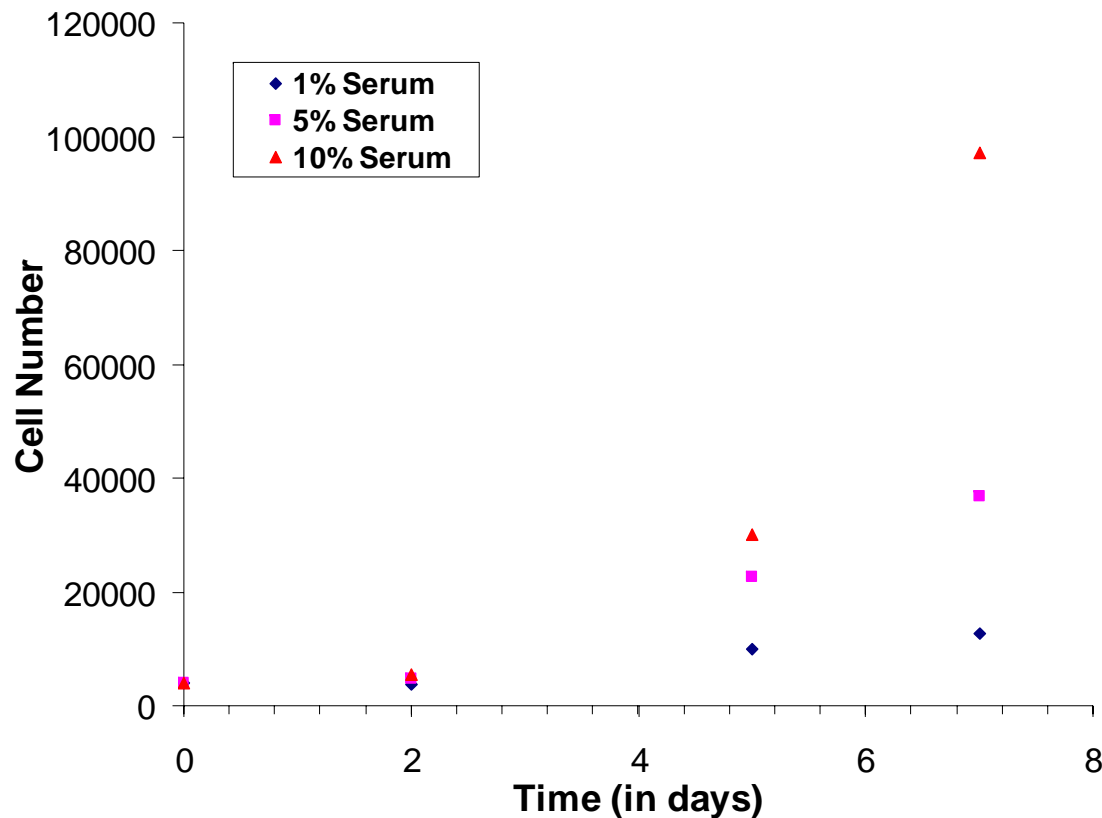
- Anti-PCNA Staining
 - Cells cultured in DMEM 1%,5%,10% FBS for 2 days, then fixed in formalin
 - Primary Anti-PCNA antibody added followed by an Anti-mouse secondary antibody
 - Hematoxylin added to stain cell nuclei
 - Cells quantified under light microscope by color
 - Red nuclei – S-phase cells (higher PCNA levels)

HDF Cell Attachment Is Increased on TC-Treated Plates



- HDF Cell Attachment is increased in TC-treated plates compared to non-TC plates at all time points (Student's t-test, $p < 0.05$)

Increased Serum Concentrations Led To Higher Observed Cell Counts



- Cell counts increased as serum concentration increased (one-way ANOVA, $p < 0.05$)

- HDF cells undergo exponential growth

- As serum level increased, doubling time decreased



Increased Serum Concentrations Led to Higher Proportion of S-Phase HDF Cells

DMEM Serum Concentration	Estimated % S-Phase Cells
1% Serum	35-55%
5% Serum	70-85%
10% Serum	80-90%

- Increased serum concentrations led to an increase in the number of observed cells in S-phase (red-stained nuclei)
- Control had 0% cells with red-stained nuclei



Conclusions

- Serum promotes HDF cell proliferation
 - Higher proportion of cells in S-phase (Anti-PCNA assay)
 - Quicker doubling times, increased rate of proliferation (Cell Proliferation assay)
- TC-treated surfaces are better than untreated for HDF cell attachment
 - Charged TC-treated surface promotes attachment