Effects of surfaces and serum concentration on HDF attachment and proliferation

YYY
February 11, 2009
BIOE 342: Laboratory in Tissue Culture
Objectives

• To determine the influence of surface modification on cell attachment
• To analyze the effects of serum concentration on cell proliferation & attachment
Cell attachment assay methods

• Human dermal fibroblasts (HDF) seeded on surface-modified polystyrene plates
  – Tissue culture treated (TC-treated)
  – Fibronectin coated (Fn-coated)
  – Untreated

• Cell density was measured 30, 75, 150, and 240 minutes after seeding using a light microscope
Surface treatment induces greater HDF attachment

* p<.05 (T-test)
Fn enables more HDF attachment

Data provided by XXX

* p<.05 (T-test)
Immunocytochemistry methods

• Anti-Proliferating Cell Nuclear Antigen (PCNA)
• HDF cells incubated for 2 days in DMEM w/ 1% antibiotic and 1%, 5%, 10% FBS (fetal bovine serum)
• After cells fixed, PCNA localized
  – 1° antibody (Ab): Anti-PCNA-mouse IgG
  – 2° Ab: Anti-mouse IgG-horseradish peroxidase (HRP)
  – HRP substrate: AEC (3-Amino-9-EthylCarbazole)
• Observed nuclei staining using light microscopy
  – Red: in S phase (HRP & AEC reaction)
  – Blue: not in S phase (hematoxylin)
More cells in S phase at higher serum concentration‡

<table>
<thead>
<tr>
<th>% FBS</th>
<th>% Confluency</th>
<th>% Red nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20-30</td>
<td>75</td>
</tr>
<tr>
<td>5</td>
<td>15-50</td>
<td>75</td>
</tr>
<tr>
<td>10</td>
<td>25-70</td>
<td>95</td>
</tr>
<tr>
<td>Control*</td>
<td>10-85</td>
<td>0</td>
</tr>
</tbody>
</table>

• With increasing serum concentration
  – Higher confluency
  – More nuclei stained red

* 3 control wells
† No statistical analysis available
Cell proliferation assay methods

• Cells plated in DMEM w/1 % FBS and 1% antibiotics

• Cells grown in 3 different growth conditions
  – DMEM w/1% FBS
  – DMEM w/5% FBS
  – DMEM w/10% FBS

• Cell concentration determined on days 0, 2, 5, and 7 using Coulter Counter
Cells exhibit exponential growth

* p<.001 (ANOVA); all are significantly different (p<.001; Tukey’s HSD)
Increasing serum concentration decreases doubling time

<table>
<thead>
<tr>
<th>Serum (% FBS)</th>
<th>Exponential rate (days(^{-1}); R(^2)*)</th>
<th>Doubling time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>0.35; 0.96</td>
<td>2.0</td>
</tr>
<tr>
<td>5%</td>
<td>0.46; 0.98</td>
<td>1.5</td>
</tr>
<tr>
<td>10%</td>
<td>0.54; 0.98</td>
<td>1.3</td>
</tr>
</tbody>
</table>

• Cell culture in 10% FBS had significantly higher population than those in 1% and 5% FBS

* Assuming all plates seeded at same concentration of cells
Serum provides essential signals for cell growth and division

- Increase FBS concentration
  - Increase number of cells in S phase
  - Increase proliferation rate
  - Reach confluency faster

- Increase serum content
  - Increase growth factors
  - Increase other cellular signals

- More cells continue through the cell cycle
- Population doubles faster
- Supported by data from cell proliferation and anti-PCNA assays