

Characterization of HDF Cell Attachment Under Varied Culture Conditions

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Objective

- Qualitatively assess HDF morphology and attachment in response to different culture conditions
 - Assess changes in attachment in response to cytotoxins
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Assessment of HDF Morphology on a Fibronectin Coated Substratum

- 40,000 HDF cells per well were seeded onto 12 wells of a 24-well plate with 4 different treatments
 - Treatment A: left uncoated
 - Treatment B: half coated by fibronectin
 - Treatment C: ring shape coat of fibronectin
 - Treatment D: coat of fibronectin covering wells
 - Cells observed under a light microscope
 - Cells rinsed with PBS then observed again
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Assessment of HDF Attachment to Different Substrata

- Three types of 24-well plates were seeded with 10,000 HDF cells per well in 12 wells and incubated
 - Untreated
 - Tissue Culture Treated
 - Fibronectin Treated*
- Sets of 3 wells from each plate were rinsed with PBS at 30, 75, 150, and 240 minutes
- Approximate cell concentrations were obtained using a light microscope

* Data courtesy of James Ramos

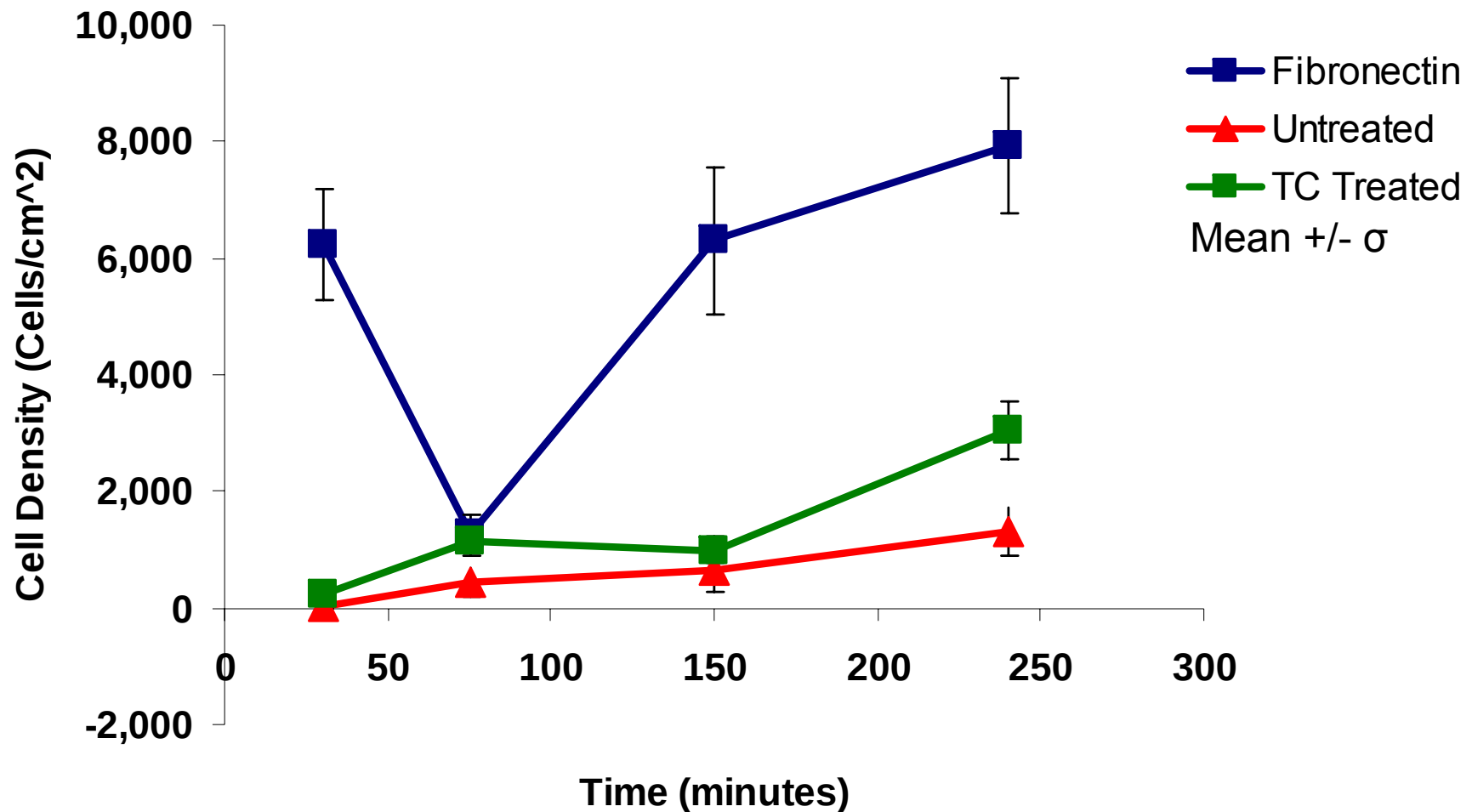
Investigating Impact of toxins on HDF Cells in Culture

- HDF cells were seeded onto nine wells of a 24-well Tissue Culture-treated plate and incubated for two days
 - Sets of three wells were given three different treatments:
 - PBS
 - PBS with two drops of Ethanol
 - Ethanol
 - Cells given live/dead fluorescent tags and viewed under a fluorescence microscope
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Fibronectin Coats Linked to Changes in HDF Morphology

- Areas lacking Fibronectin coat
 - Rounded cells
 - Well-defined nucleus
 - Nucleus comprises most of visible cell area
 - Areas with Fibronectin coat
 - Cells with fibrous appearance
 - Pseudopodia extending
 - Less well-defined nucleus comprising less visible cell area
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Fibronectin Coat Leads to Higher Ultimate Cell Density



Fibronectin Linked to Higher Cell Attachment After Four Hours

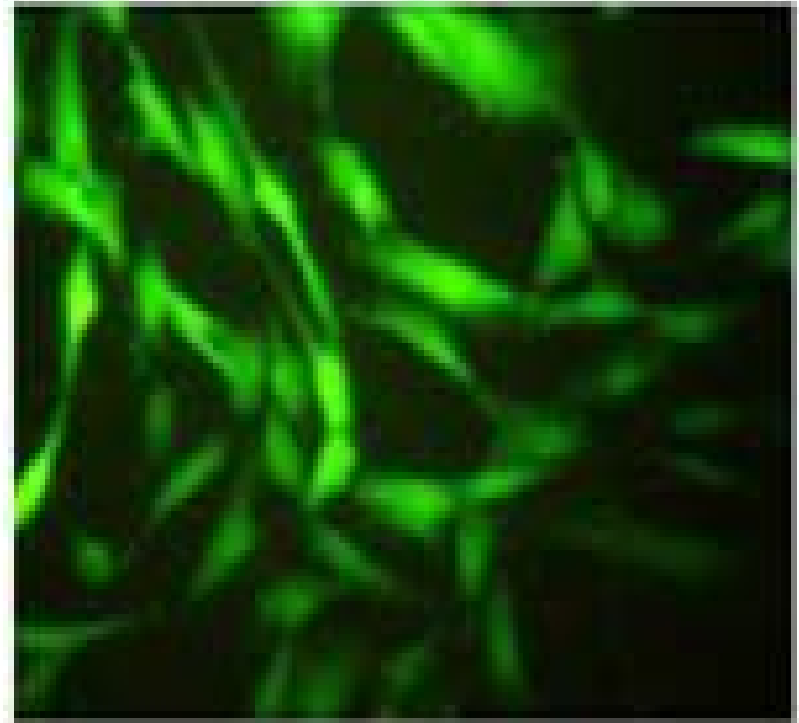
- Assume rinsing wells with PBS removes poorly attached cells
 - Remaining cell density comprises well-attached cells
 - Fibronectin-coated substrata display higher cell densities at four hours than:
 - TC-treated substrata ($n = 3$, $p = .0023$)
 - Untreated substrata ($n = 3$, $p = .0008$)
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Changes in Cell Morphology Linked to Changes in Cell Attachment

- HDF cells have a distinct morphology when cultured on a Fibronectin substratum
 - Fibrous with pseudopodia
 - HDF cells have higher attachment to a Fibronectin substratum compared to other substrata
 - The morphology of cells on Fibronectin may be associated with increased cell attachment on Fibronectin
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Cell Viability Described by Color of Fluorescence

- HDF cells appear different under fluorescence if they are alive/dead:
 - Green if alive
 - Red if dead
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Example of Live/Dead fluorescence borrowed from Hoerter et al. Effect of UVA Fluence Rate on Indicators of Oxidative Stress in Human Dermal Fibroblasts, Journal of Biological Science 2008; 4:63-70

Note that red cells are dead, green cells are alive

Cell Detachment Qualitatively Relates to Viability

- Wells without ethanol appear almost entirely green
 - ~5% cell detachment
 - Wells with ethanol drops appear with patches of red towards well equators
 - ~10% cell detachment
 - Wells with entirely ethanol are completely red
 - ~10-15% cell detachment
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Live HDF Cells on Fibronectin Substrata Attach Well

- In cultures of HDF cells where attachment is desired, use a Fibronectin substratum and try to ensure maximum cell viability

