Attachment and Viability of Human Dermal Fibroblasts

XXX, Rice University

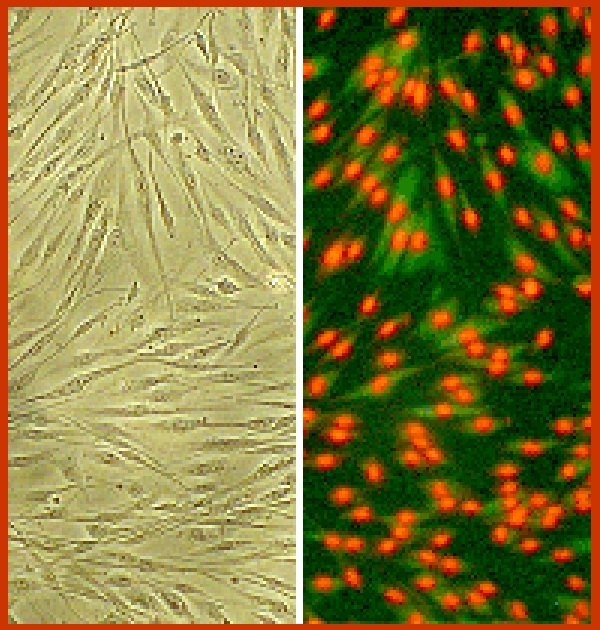

Diagram 1:

Left: attached HDF cells, Right: HDF cells seen under fluorescent microscope

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Laboratory in Tissue Culture

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Objectives

- To both visualize and quantify attachment of HDF cells in various media and surfaces

    + Fibronectin Attachment Assay

    + Quantitative Cell Attachment Assay

- To analyze HDF cell viability via fluorescent microscopy and to examine toxicity of cells

    + Live/Dead Fluorescent Assay

Experimental Methods: Fn Attachment Assay

Materials:

-non-TC treated 24-well paint
- Fibronectin (Fn)
- HDF cells (50,000 cells/mL)
- PBS with 10 mg/mL BSA
-PBS
- DMEM (no serum or antibiotics)

Test Conditions:

- A: No Fn
B: Half Fn
- C: “X” design with Fn
- D: All Fn
- 3 wells each

Methods:

1. Paint Fn in all the test wells as designated by their test condition and PBS in the control wells, incubate 30 minutes then aspirate and rinse wells with PBS + BSA 3 times
2. Seed cells (mixing cell suspension every 3 wells), incubate 2 hours and check plate under microscope
3. Aspirate media, rinse with PBS
4. Add 250 µL PBS to each well and examine under microscope

Results: Fn Attachment Assay

Condition A: <10 cells visible, cells were round and very bright under the microscope

Condition B: The left side (half painted with Fn) was 45% confluent and cells were randomly spread; the right side had <5 cells visible

Condition C: ¾ of "X" covered in cells though cells were also visible around the design; cells appear darker on the "X"

Condition D: Well was 80% confluent, evenly spread and somewhat globular

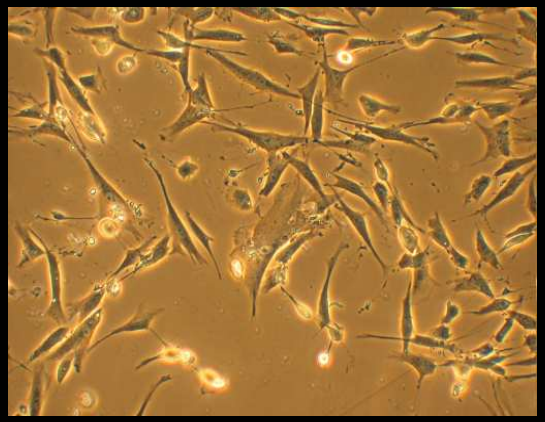

Diagram 2:

Mammalian fibroblast on a Fn treated surface

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Summary:

Fibronectin aids cell attach to non-tissue culture treated surfaces. Fn attached cells appear more globular in shape and do not have the "bright ring" visible around cell under light microscopy using a filter.

Experimental Method: Quantitative Cell Attachment

Materials:

- Polystyrene 24 well plates:
  - +TC Treated
  - + non-TC Treated
  - + Fn Coated
- HDF cells (10,000 cells/mL)
- DMEM (10% serum and 1% antibiotic)
- Light Microscope

Test Conditions:

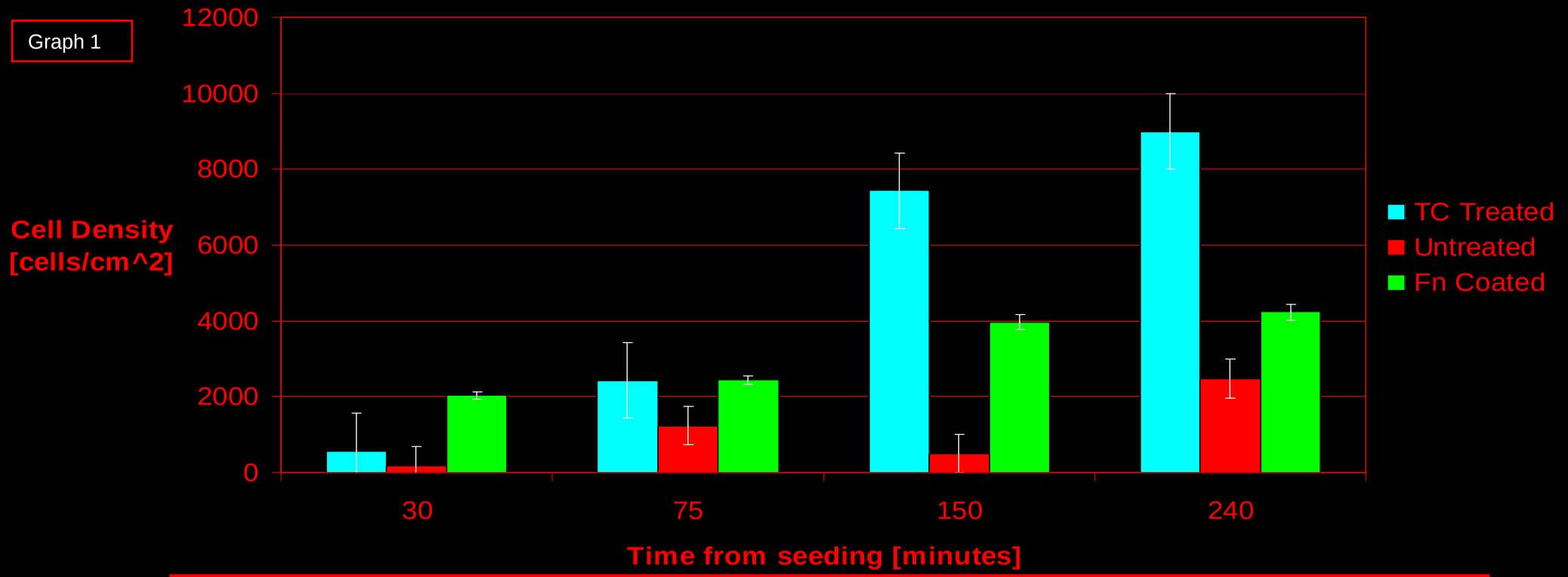
- 4 time points to be taken at 30, 75, 150, and 240 minutes
- 2 wells at each time point on each plate

Method:

1. Dilute cells in DMEM and seed1 mL in all test wells (36 total) mixing every 3 wells
2. After seeding, incubate all wells and begin the 4 hour count
3. Do the following to each well of each plate on the proper time point for all 4 test points:
   - i. Aspirate and rinse with PBS 3 times
   - ii. Add 1 mL PBS to each well
   - iii. Count cells under microscope and observe morphology, shape, and "spreadness" of cells

Results: Quantitative Cell Attachment

Graph 1



Summary:

Cells attached best on TC treated polystyrene, then FN coated plates, then, the untreated plate. With each time point, there were more cells attached and each cell was more spread on the surface.

Comparative Results

- Fn Attachment was a qualitative experiment and resulted in firmly attached cells that are not simply round in shape
- Quantitative Cell Attachment resulted in more accurate concentration calculations and also more precise because Fn was evenly coated on the well and not painted on by a student

Experimental Method: Live/Dead Fluorescence Assay

Materials:

- TC Treated 24-well plate
- HDF (1:3 diluted from 1:10)
- DMEM (10% serum and 1% antibiotic)
- PBS
- Ethanol
- Live/Dead reagent (dye)

Test Conditions

- A: Live cells
- B: Dead cells
- C: Live and Dead cells
- 3 wells each

Methods:

1. After diluting cells to 1:3 then seed 9 test well with 1mL cell suspension, mixing cells every 3 wells, incubate 2 nights and observe
2. Aspirate and Rinse 2 times
3. Add 250 µL PBS and 100 µL dye to A, 250 µL ethanol and 100 µL dye to B, and 250 µL ethanol, 2 drops ethanol, and 100 µL dye to C (in that order)
4. Loosely but completely cover plate with foil and incubate in hood for 30 minute
5. Observe cells under light and fluorescent microscopes

Results: Live/Dead Fluorescence Assay

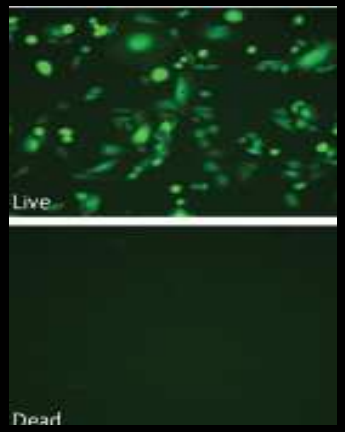


Diagram 3:

Mammalian cells in condition A (upper) and B (lower) seen through light microscopy

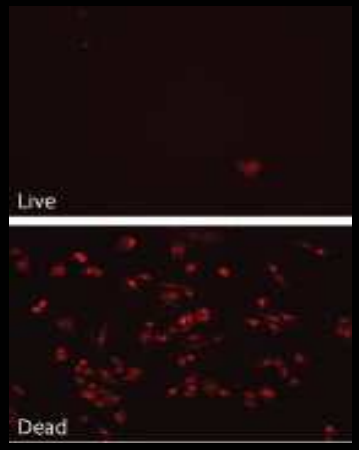


Diagram 4:

Mammalian cells in condition A (upper) and B (Lower) under a fluorescent microscope

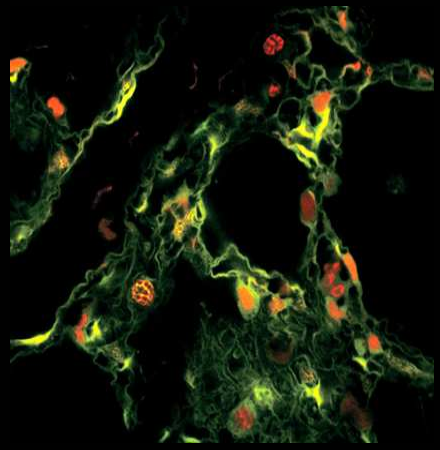


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Diagram 5:

Mammalian cells in condition C under a fluorescent microscope

http://www.activemotif.com/images/products/toxcount_fluorescent_micro.jpg

Summary:
Ethanol is toxic to cells and more ethanol kill more cells. Live cells can be visually differentiated from dead cells easily using this assay, which allows live cells to appear green and dead cells red.

Summary

- Both attachment tests demonstrated that cells attach better over time regardless of the surface and these attached cells were globular in shape with viewable extensions and rounded bodies

- Viable cells are easily seen with a using fluorescent microscopy and are attached to the given surface

Diagram 6:

Viable mammalian cells firmly attached to the surface

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