

# Human Dermal Fibroblasts *in vitro*: Attachment and Proliferation

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# Overall Experimental Objectives

- To *qualitatively* observe Human Dermal Fibroblasts (HDF) cell morphology & attachment to fibronectin-coated surface
- To *quantitatively* measure HDF cell attachment to fibronectin coated,  $\gamma$ -irradiation-treated or untreated surfaces
- To *quantitatively* evaluate the effects of 1%, 5%, and 10% serum on proliferation of HDF cells

# Fn Promoting Attachment: Qualitative Assay Methods

- *Experimental Variables*: Amount & area of coating
- Fibronectin (Fn) painted onto non TC-treated well plates & incubated
- Four well conditions:
  1. Entire Fn coating
  2. Half painted Fn
  3. Design painted Fn
  4. No Fn painted
- HDF cell seeded, incubated for 2 hrs & washed
- Observations recorded using light microscope

# Morphology & Attachment of HDF Cells to Fn Coating *before* Wash

|                          | Morphology                  | Attachment                       |
|--------------------------|-----------------------------|----------------------------------|
| <b>Entire Fn Coating</b> | Many spread, few rounded    | All over well surface            |
| <b>Half Painted Fn</b>   | Some spread, others rounded | On painted half & center of well |
| <b>Design Painted Fn</b> | Some spread, others rounded | Sporadic on design areas         |
| <b>No Fn</b>             | <i>All rounded</i>          | <i>None</i>                      |

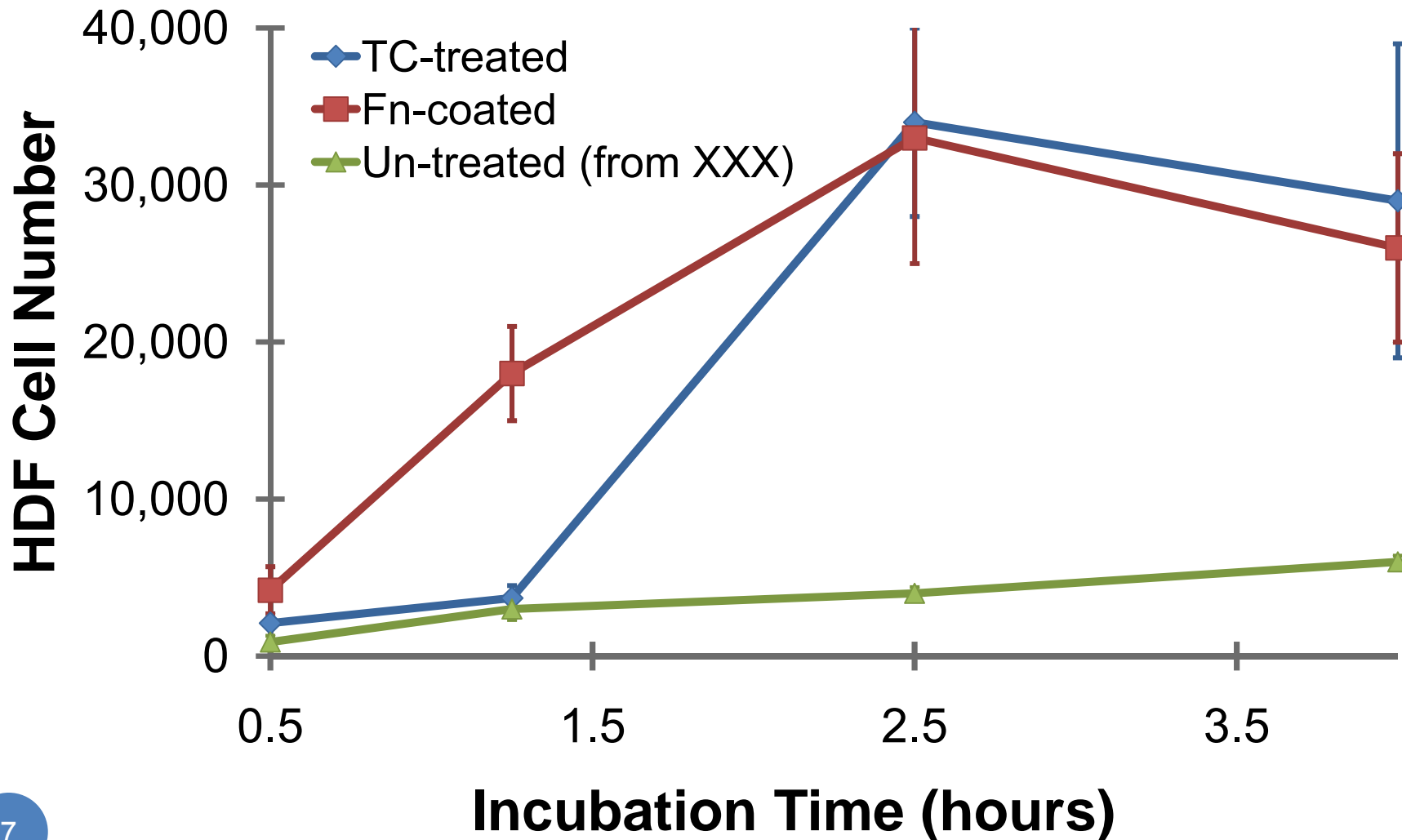
# Spread Morphology & Evident Attachment of HDF cells to Fn Coating *after Wash*

|                   | Morphology              | Attachment                                   |
|-------------------|-------------------------|--|
| Entire Fn Coating | <i>Spread</i>           | Center of well, <i>none floating</i>         |
| Half Painted Fn   | <i>Spread</i>           | On painted half, <i>none floating</i>        |
| Design Painted Fn | <i>Spread</i>           | On painted design area, <i>none floating</i> |
| No Fn             | <i>No cells present</i> |  |

# Fibronectin Promoting Attachment in Less Time: Quantitative Assay Methods

- *Experimental Variables*: Surface treatment & incubation time
- Three pre-treated 24 well plates
  1.  $\gamma$ -irradiation(TC)
  2. Fn-coated
  3. Untreated
- HDF cells seeded at 10,000 cells/mL & incubated for 0.5, 1.25, 2.5, & 4 hrs
- After rinsing, counted attached cells in 10 X 10 grid with a light microscope, 10X objective

# Fibronectin-Coated Plate Allows for Increased Cell Attachment at 1.25 Hours



# HDF Cell Attachment with Fibronectin: Qualitative & Quantitative Assays Synthesis

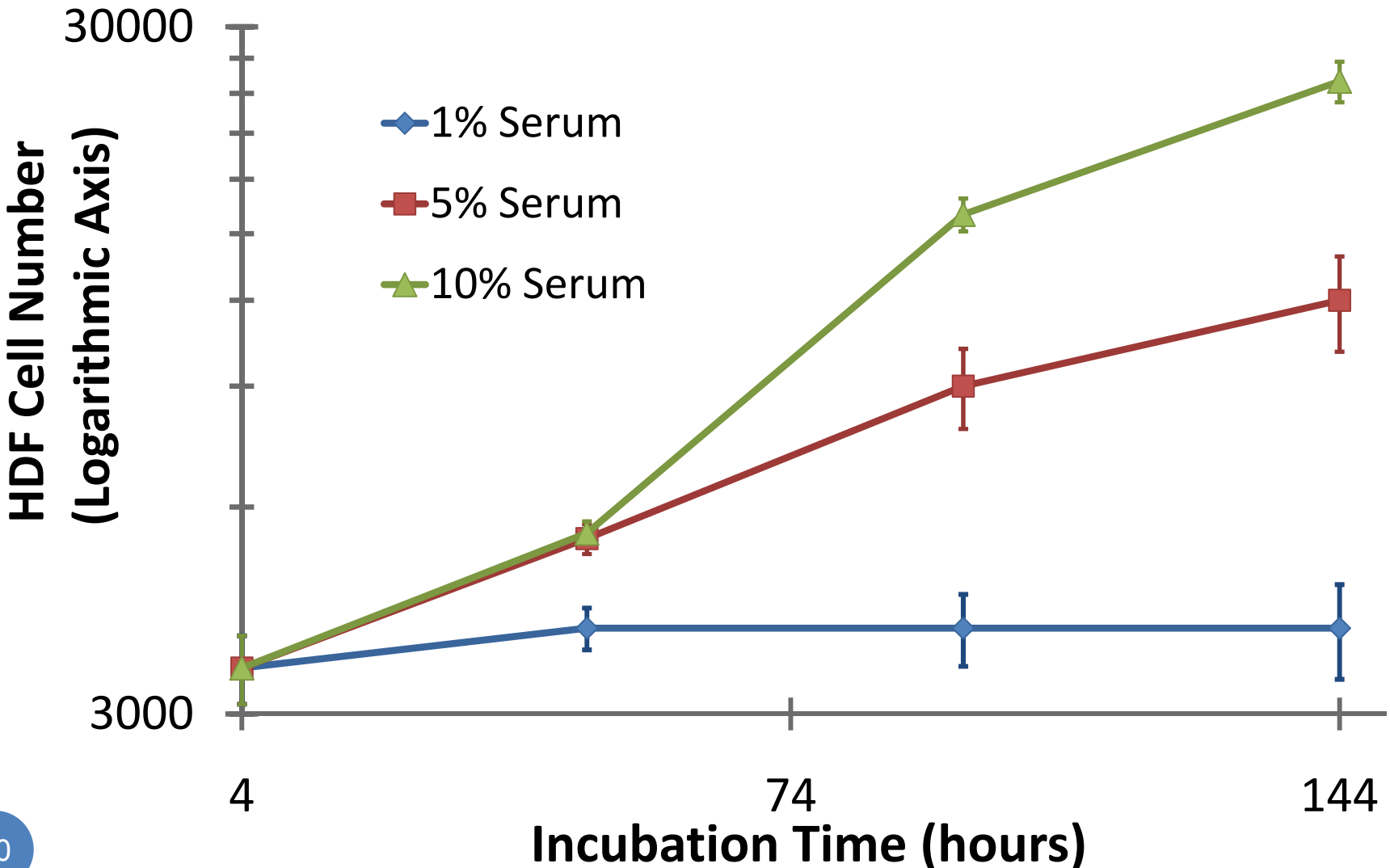
- Qualitative Assay:
  - HDF cell attachment & spread cell morphology on Fn coating *only*
- Quantitative Assay:
  - Few HDF cells attach to un-treated surface
  - HDF cells attach to Fn-coated surface in half the time for  $\gamma$ -irradiated surface
- Fn suitable protein for coating HDF cell attachment surfaces & achieve attachment within 1.25 hours



# HDF Cell Proliferation with Increasing Serum Percentages: Assay Methods

- *Experimental Variables:* Serum percentage in DMEM & incubation time
- HDF cells seeded to TC-treated wells with DMEM and 1, 5, or 10% serum
- Cells trypsinized and rinsed after 4, 48, 96, & 144 hours
- Number of cells per well determined with Coulter Counter

# Proliferation of HDF Cells in 5 & 10% Serum



# Statistical Differences Among HDF Cell Numbers in 1, 5 & 10 Serum Percentages After 144 hours

|                | 1% Serum        | 5% Serum           | 10% Serum        |
|----------------|-----------------|--------------------|------------------|
| <b>4 hrs</b>   | 3,500±400       | 3,500±400          | 3,500 ± 400      |
| <b>48 hrs</b>  | 4,000±300       | 5,400±300          | 5,500 ± 200      |
| <b>96 hrs</b>  | 4,000±500       | 9,000±1,000        | 16,000 ± 900     |
| <b>144 hrs</b> | 4,000±600*<br>^ | 12,000±2,000^<br>~ | 25,000 ± 2,000*~ |

\*^~P < 0.05

# Estimated Doubling Time

- HDF cells incubated in 1% serum experienced no exponential growth
- HDF cells incubated in 5% & 10% serum experienced exponential growth
- HDF cells' doubling times:
  - 5% serum ~70 hrs
  - 10% serum ~ 50 hrs
- The largest cell number and a decreased doubling time of 10% serum suggests increased proliferation

# Conclusions for HDF Cell Attachment and Proliferation

- Fibronectin promotes spread morphology for attachment of HDF cells to non-treated surfaces
- Fibronectin can be coated on non-treated surfaces to attach HDF cells if only 1 hr incubation time needed
- HDF cells achieve 50 hr doubling time & exponential growth when incubated in DMEM with 10% serum
- HDF cells cannot proliferate when incubated in DMEM with 1% serum