HDF Cells Survival and Activity In Vitro

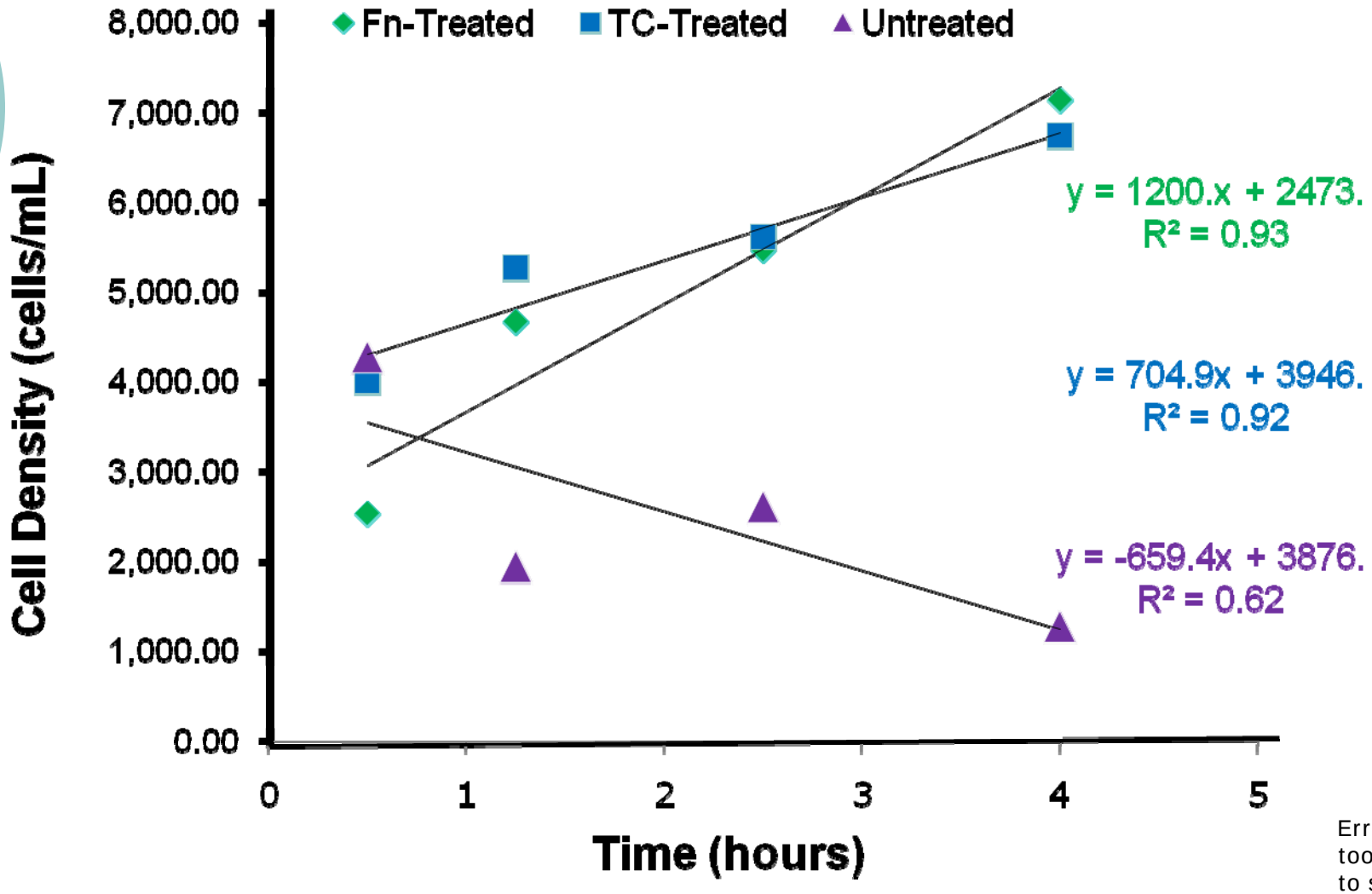
Purpose

- Study attachment of HDF (Human Dermal Fibroblast) cells to different surfaces
  - Quantitative Cell Attachment Assay
- Determine the relationship between absorbance, cell concentration, and viability
  - MTT Viability Test
- Asses cell viability
  - Live/Dead Fluorescence Assay

Cell Attachment Assay Methods

- Cells seeded at 10,000 cells/mL in DMEM with 10% serum
- Attachment to three surfaces studied:
  - TC -Treated Polystyrene
  - Untreated Polystyrene
  - Fibronectin (Fn)-Coated Polystyrene
- Cells rinsed with PBS to remove unattached cells
- Attached cells counted with Coulter Counter at:
  - 30 min
  - 1 hr and 15 min
  - 2 hrs and 30 min
  - 4 hrs

Cell Attachment Dependent on Surface Treatment



Untreated Data obtained from XXX

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Results of Cell Attachment

- Cells adhered to:
  - Fn coated surface
    - Contains ligands/proteins to promote adhesion
  - TC-treated plates
    - Charged surface, wettable
  - Untreated surface discouraged cell attachment
  - Of the three tested conditions Fn-coated had largest final cell density
  - After 4 hours, 50 % cell attachment seen on Fn-coated surface

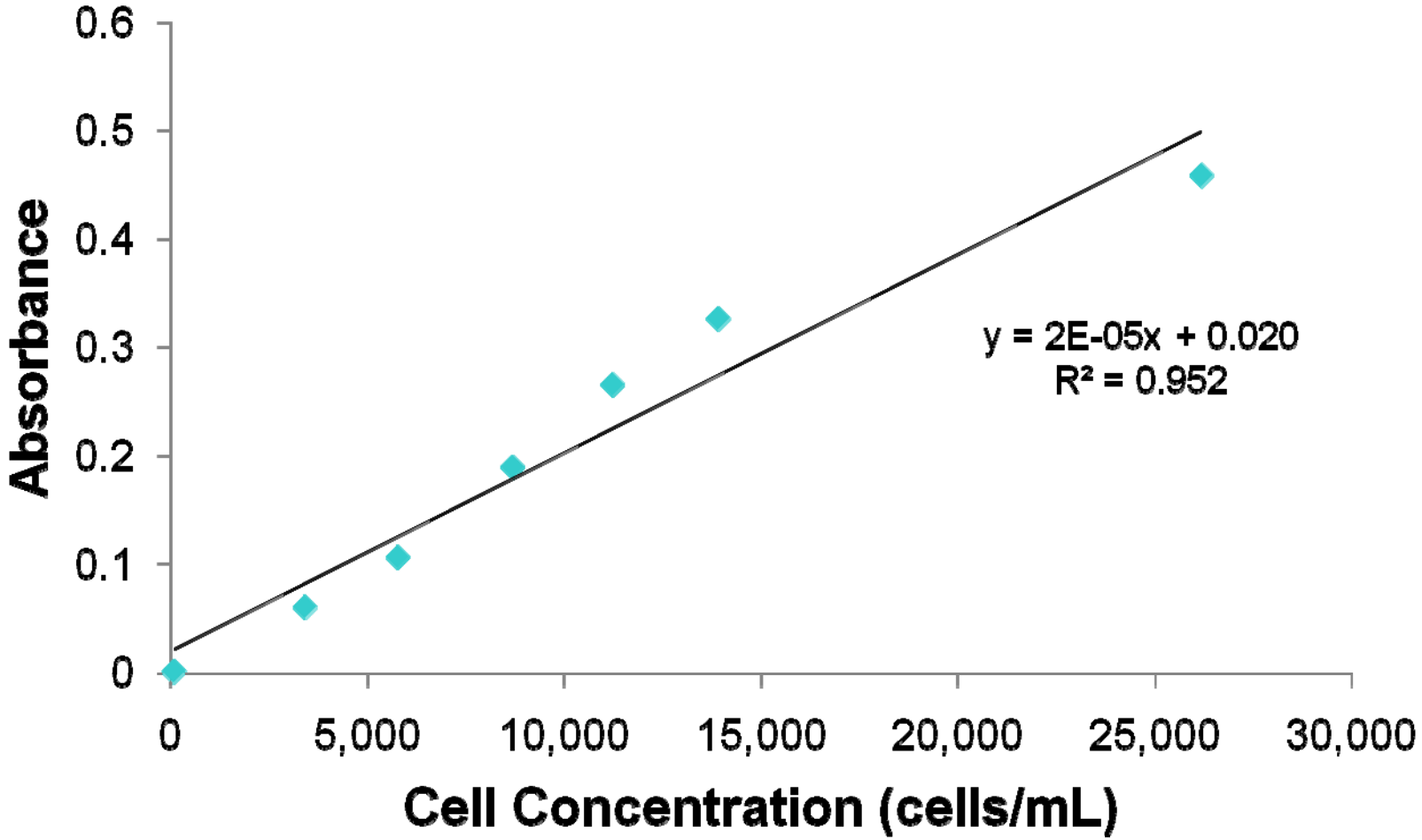
MTT Viability Test Methods

- Cells seeded at 6 different densities:

Well	1 (Stock)	2	3	4	5	6	Control
Cell Concentration (cells/mL)	50,000	33,500	25,000	16,700	8,330	4,170	0

- Incubated in 10% serum in DMEM for 2 days
- Seeding densities confirmed with Coulter Counter
- Incubated with dye solution for 2 hours before solubilization/stop solution added
- Absorbance recorded at 570nm on Genesys 10UV Spectrophotometer

Absorbance Increases With Higher Cell Concentrations



MTT Dye is a Good Indicator of Cell Health

- MTT indirectly measures:
  - Metabolic cell activity
  - Cell health
- Higher absorbance correlates to greater number of living cells.
  - The more viable the cells are, the more dye metabolized
- Shows overall health of cellular environment

Live/Dead Assay Methods

- Cells incubated for 2 days with 10% serum in DMEM
- Treatment Conditions:

Condition A	**PBS and Dye**
Condition B	**Ethanol and Dye**
Condition C	**PBS, 2 drops of Ethanol and Dye**

- Live cell membranes stained with calcein acetoxymethyl (calcein AM)-green stain
- Dead cell nuclei stained with Ethidium homodimer-1 (EthD-1)- red stain
- Nikon Fluorescent Microscope used to observe cells

Ethanol is Toxic to Cells

Test Conditions	Observations	Results
PBS	**Cell Membrane stained green**	**100% of cells viable**
Ethanol	**Cell Nucli stained red**	**All Cells Dead**
PBS and 2 drops Ethanol	**Mostly green, 20% nuclei stained red**	**80% of cells viable**

- Red stain indicates dead cells
- Green stain indicates living cells
- Ethanol is toxic, it dissolve lipids out of cell membrane.

MTT and Live/Dead Assays Both Measure Cell Viability

- Both rely on cells chemically changing indicator dye
- MTT indirectly measures cell viability
  - Measures absorbance of dye metabolized by healthy cells
- Live/Dead directly measures cell viability
  - Staining shows definitively if cells are dead or alive
- Unlike Coulter Counter both assays are discriminatory towards dead cells

Conclusions

- Cell attachment dependent on surface treatment
  - Cells adhered to Fn coated and TC-treated surfaces
  - Untreated surface discouraged cell attachment
- Absorbance related linearly to cell concentration
- Ethanol is detrimental to cells
- MTT and Live/Dead Assays are complimentary in determining cell viability